European Journal of Neuroscience, Vol. 13, pp. 1228–1238, 2001

Impairments in visual discrimination learning and recognition memory produced by neurotoxic lesions of rhinal cortex in rhesus monkeys

Mark G. Baxter[1] and Elisabeth A. Murray[2]
[1]Department of Psychology, Harvard University, 906 William James Hall, 33 Kirkland Street, Cambridge, MA 02138, USA
[2]Laboratory of Neuropsychology, National Institute of Mental Health, 49 Convent Drive, Bethesda, MD 20892, USA

Keywords: discrimination learning set, ibotenic acid, primary reinforcement, reversal learning

Abstract

Much work on the cognitive functions of the primate rhinal (i.e. entorhinal plus perirhinal) cortex has been based on aspiration lesions of this structure, which might disrupt fibres passing nearby and through the rhinal cortex in addition to removing the cell bodies of the rhinal cortex itself. To determine whether damage limited to the cell bodies of the rhinal cortex is sufficient to impair visual learning and memory, four rhesus monkeys (*Macaca mulatta*) were preoperatively trained on a battery of visual learning and memory tasks, including single-pair discrimination learning for primary reinforcement, single-pair discrimination reversals, concurrent discrimination learning and reversal, and delayed matching-to-sample. Following acquisition of these tasks and a preoperative performance test, ibotenic acid was injected bilaterally into the rhinal cortex, and the monkeys were retested. Consistent with the results of studies using aspiration lesions, the monkeys were impaired on single-pair discrimination learning as well as recognition memory performance postoperatively, although reliable reversal learning impairments were not observed. The magnitude of postoperative impairment in discrimination learning was not correlated with the magnitude of postoperative impairment in recognition memory, suggesting a possible dissociation between these functions within the rhinal cortex. The correspondence of behavioural deficits following aspiration and neurotoxic lesions of the rhinal cortex validates the attribution of various cognitive functions to this structure, based on the results of studies with aspiration lesions.

Introduction

The rhinal cortex, composed of the entorhinal and perirhinal cortices, is a multimodal cortical area located on the ventromedial surface of the temporal lobe, subjacent to the amygdala and rostral hippocampus. Behavioural studies of monkeys with lesions of the rhinal cortex or its components have implicated this cortical region in a number of cognitive processes, including visual discrimination learning (Buckley & Gaffan, 1997; Baxter *et al*., 1999), recognition memory (Meunier *et al*., 1993; Eacott *et al*., 1994; Buffalo *et al*., 1999), cross-modal association memory (Parker & Gaffan, 1998; Goulet & Murray, 2001), and object identification (Buckley & Gaffan, 1998b; Murray & Bussey, 1999). One current view of perirhinal cortex function is that it forms the kernel of a neural system specialized for acquiring knowledge about objects (for review, see Murray, 2000). Such a role is consistent with the anatomical connections of the rhinal cortex; in addition to receiving highly processed visual information from the ventral visual stream (Suzuki & Amaral, 1994), this region also receives information from other sensory modalities (Friedman *et al*., 1986; Suzuki & Amaral, 1994; Suzuki, 1996), creating a specialization within this region for object perception and memory (Murray & Bussey, 1999).

It is now well established that many of the cognitive functions originally attributed to medial temporal lobe structures located deep to the rhinal cortex, namely the amygdala and hippocampus, can be attributed with confidence to the overlying rhinal cortex instead (Murray, 1992, 2000; Baxter & Murray, 2000). The original misattribution of function appears mainly to be due to disruption of fibres passing nearby or through the amygdala, together with direct damage to some of the underlying cortex, that occurred in association with aspiration removals of the amygdala and hippocampus. Only with the recent application of more selective lesion techniques has it become clear that the rhinal cortex is more important for memory than previously recognized.

Unlike the amygdala and hippocampus, the rhinal cortex is located on the surface of the brain and therefore can be accessed directly for aspiration lesions. Thus, the likelihood of misleading results due to disruption of fibres of passage would seem remote in this situation. Nevertheless, as alluded to earlier, striking dissimilarities between behavioural effects of aspiration and electrolytic vs. neurotoxic lesions of the amygdala have been reported (Dunn & Everitt, 1988; Málková *et al*., 1997). Such findings suggest that it would be prudent to confirm any behavioural impairments observed following aspiration lesions of the rhinal cortex with studies using neurotoxic lesions. Only in this way can one ensure that deficits observed are due to damage to cell bodies within the rhinal cortex, rather than damage to fibres of passage moving nearby or through the rhinal cortex.

The present study was conducted with this in mind. Rhesus monkeys were preoperatively trained on a battery of visual learning and memory tasks, given neurotoxic lesions of the rhinal cortex, then retested to assess deficits associated with damage to the cell bodies of

Correspondence: Dr Elisabeth A. Murray, as above.
E-mail: eam@ln.nimh.nih.gov

Received 29 September 2000, revised 12 January 2001, accepted 18 January 2001

the rhinal cortex. This study had two important features. First, as already indicated, a neurotoxin (ibotenic acid) was used to damage the rhinal cortex, rather than direct aspiration. Second, the testing of monkeys on multiple tasks, some thought to rely on rhinal cortex, permitted a direct comparison of deficits in different cognitive domains after rhinal cortex damage.

Materials and methods

Subjects

Four experimentally naive rhesus monkeys (*Macaca mulatta*), all males, were used. They weighed 4.3–5.2 kg at the beginning of the study, were housed individually in rooms with automatically regulated lighting (12 h light : 12 h dark, lights on at 07.00 h), and were maintained on primate chow (#5038, PMI Feeds Inc., St Louis, MO, USA) supplemented with fresh fruit and peanuts. Water was always available in the home cage. These experiments were approved by the NIMH Animal Care and Use Committee.

Apparatus and materials

The monkeys were trained in an automated apparatus consisting of an IBM-compatible computer connected to a colour monitor fitted with a touch-sensitive screen (Microtouch Systems, Woburn, MA, USA) and an automatic pellet dispenser (BRS/LVE, Laurel, MD, USA). A large set of complex visual stimuli was used. Each stimulus consisted of two different ASCII characters of two different colours and two different sizes superimposed. These stimuli were created by an algorithm reported previously (Murray *et al*., 1993); stimuli of this type were used for all the tasks described in this study.

For each test session, the monkey was seated in a primate chair inside a testing cubicle. The monkey's head was approximately 230 mm from the monitor, and the monkey's arms were free to reach toward any part of the screen. A visual stimulus could appear in one of three locations on the monitor, either on the left side or the right side of the screen, 90 mm from the centre, or in the centre. Rewards were delivered through a copper tube into a food cup located directly below the centre of the monitor. The food rewards were banana-flavoured pellets (190 mg; Noyes, Lancaster, NH, USA). A closed-circuit television camera enabled the monkey to be observed by the experimenter during the test sessions.

Preoperative testing

Pretraining

The monkeys were first placed on an autoshaping task through which they learned to touch the screen to obtain a reward. A single stimulus appeared on either the left or right side of the monitor screen. If the monkey touched the stimulus, the stimulus disappeared and a banana pellet was delivered. If the monkey did not touch the stimulus, after 10 s the stimulus disappeared and a banana pellet was delivered. Thirty novel stimuli were presented on a variable-interval schedule with a mean inter-trial interval of 2 min. The left–right position of the stimuli on the screen varied randomly across trials. The criterion for completing this stage of training was 2 consecutive days with one or more responses. If the monkey failed to make any responses after 4–5 days in this procedure, it was advanced to the next phase of pretraining and manually shaped to touch the screen. Then the monkeys were given a second shaping task, one in which the delivery of reward was contingent on a response to the stimulus. As before, a single visual stimulus appeared on either the left or right side of the screen, but now the stimulus remained on the screen until the monkey responded to it by touching it. When the monkey touched the stimulus, the stimulus disappeared and a pellet was delivered. After a 6-s inter-trial interval, the process was repeated with another stimulus, and so on. In this manner, 100 novel stimuli were presented in each session. The monkeys were required to complete two sessions on this task before advancing to the main experiment.

Discrimination learning set

In this task, each monkey was required to solve new single-pair visual discrimination problems within sessions, as described later. On each trial, two stimuli were presented simultaneously on the screen, one each on the left and right sides of the screen. One stimulus was arbitrarily designated correct, and the other incorrect. If the monkey touched the correct stimulus, both stimuli disappeared and a banana pellet was delivered. If the monkey touched the incorrect stimulus, both stimuli disappeared and no reward was given. There was no visual feedback provided to the monkey after selecting a stimulus, and no correction for errors; only delivery (or not) of a banana pellet signalled which stimulus was correct. The same problem was presented for 20 consecutive trials. After 20 trials, a new problem was presented, again for 20 trials. This procedure was repeated until a total of five problems had been presented in each session. The interval between trials within a problem was 5 s; the interval between problems was 10 s. The left–right location of the correct stimulus followed a random order. The monkeys were tested at the rate of five problems per session, one session per day, for a total of 45 sessions.

For consistency with previous studies, we have retained the designation 'discrimination learning set' for this type of rapid, single-pair learning. We note, however, that our intention is to examine the effects of rhinal cortex lesions on the rate of acquisition of discrimination problems once a learning set has been established, not to measure the effects of rhinal cortex lesions on the development or maintenance of a discrimination learning set.

Single-pair discrimination reversals

After the monkeys completed training on a discrimination learning set, they were required to learn reversals of discrimination problems within sessions. Two novel problems were presented serially within each session. Once a problem was learnt to a criterion of 38 correct responses in 40 consecutive trials, the reward contingencies were reversed; responses to the previously correct stimulus did not result in reinforcement, whereas responses to the previously incorrect stimulus did. Each reversal was learnt to a criterion of 18 correct responses in 20 consecutive trials. Each session thus included learning of problem 1 followed by reversal of problem 1, then learning of problem 2 and, finally, reversal of problem 2.

Each session of the 'reversal' task was preceded by two sessions of the regular discrimination learning set task (i.e. no reversal), with the parameters changed so that only two problems were given and each problem appeared for 60 consecutive trials. This was done to prevent the monkeys from using the extended presentations of a single problem in the reversal task as a cue that a change in reward contingencies was imminent. A total of four sessions of reversal training were given, for a total of eight problems learned and reversed.

Concurrent discrimination learning and reversal

The monkeys were next tested on a set of discrimination problems that were learned across sessions. The eight-pair, concurrent discrimination learning task described by Zola and colleagues (e.g. Buffalo *et al*., 1999) was employed, but in an automated rather than manual format. Eight pairs of discriminanda, each composed of a pair

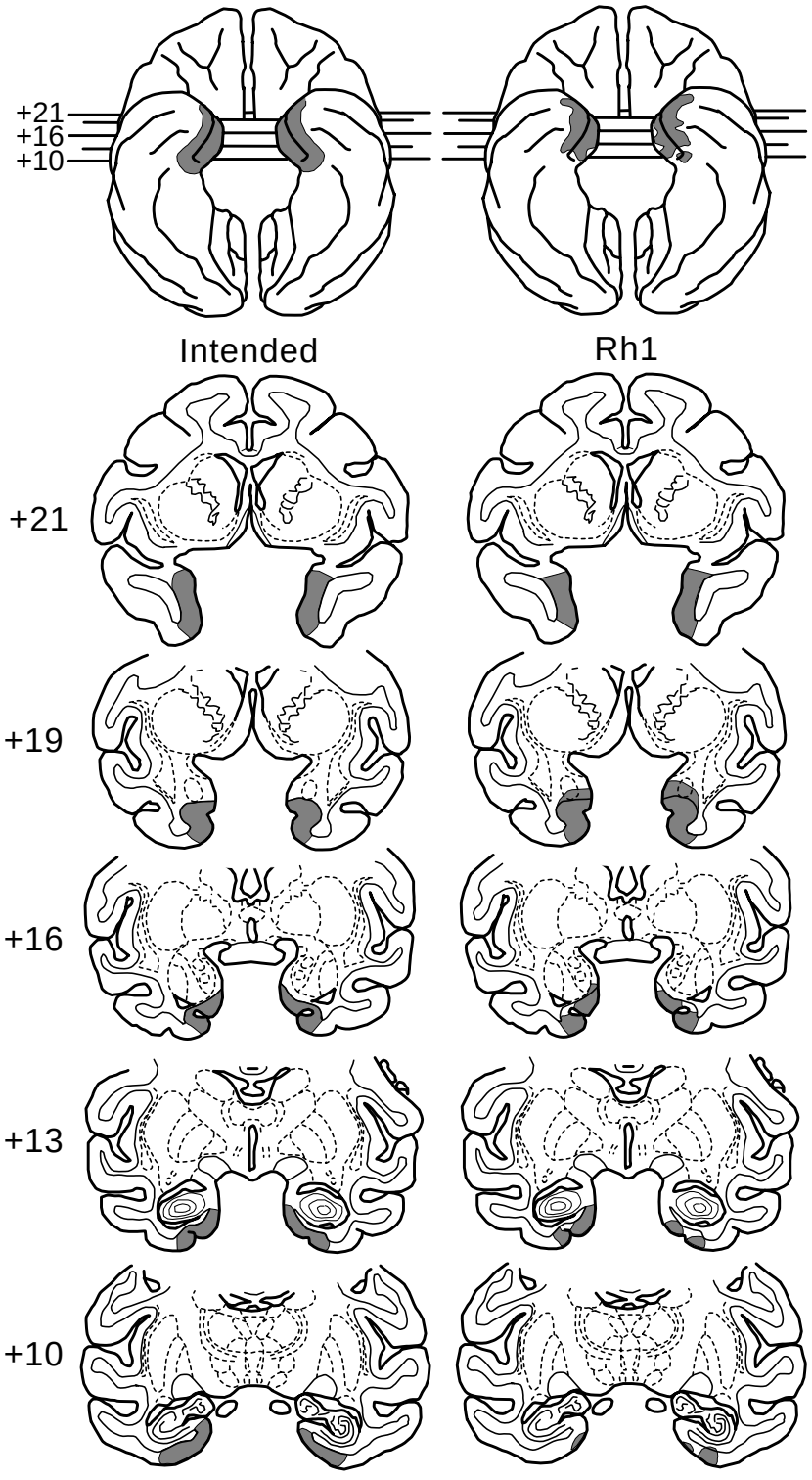
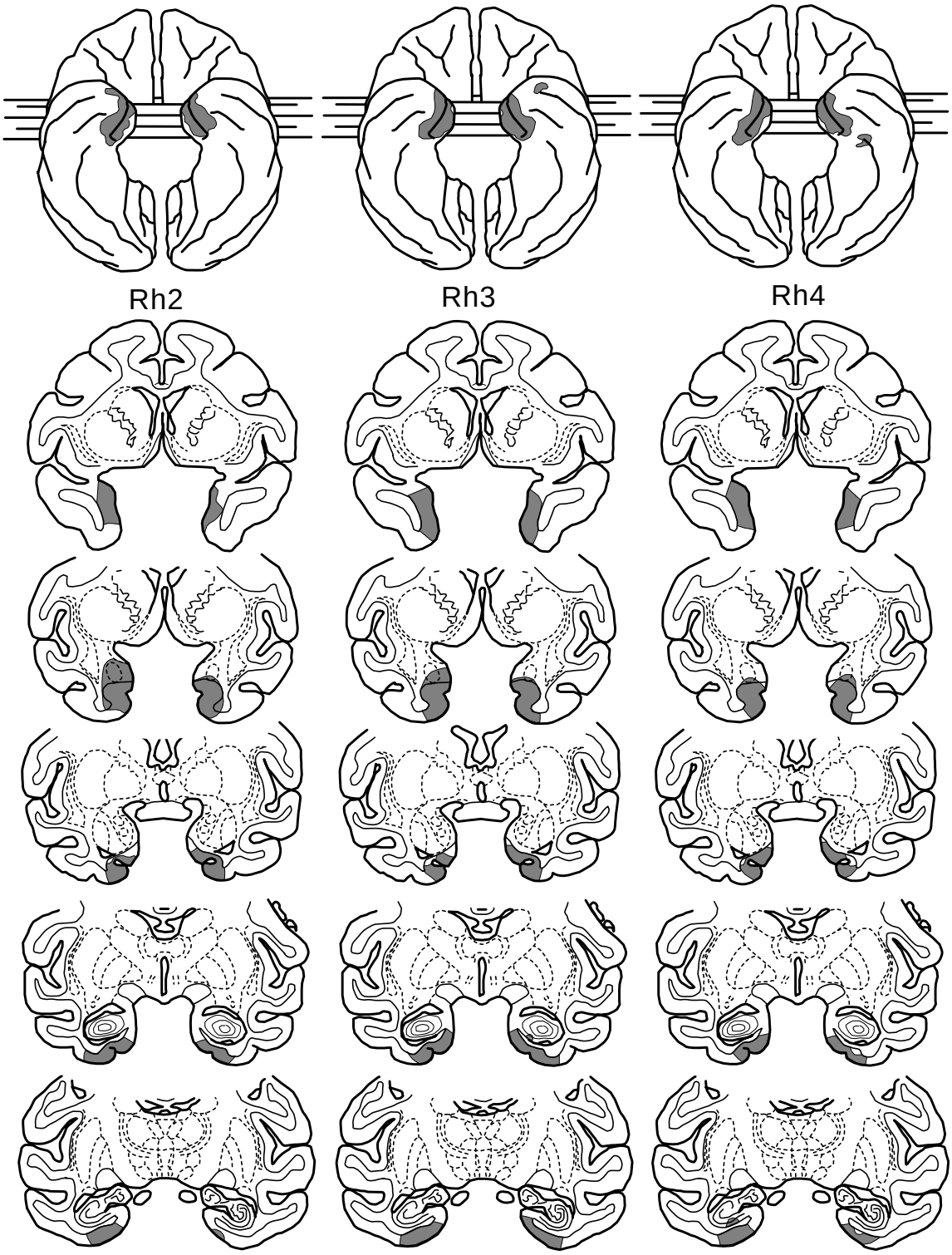


FIG. 1. Intended lesion and plots of the neurotoxic rhinal cortex lesions (shaded regions) in cases Rh1–4, shown on ventral surface views (top) and coronal sections (below) from a standard rhesus monkey brain. The intended lesion is shown in the leftmost column. The ventral views for Rh1–4 show reconstructions of the extent of the rhinal cortex lesions and are reversed to aid in matching to the individual sections (i.e. the left hemisphere is on the left); the positions of the stereotaxic levels illustrated in the coronal sections are also indicated. The numerals to the left of the coronal sections indicate the distance in millimetres from the interaural plane. Compare and contrast with the photomicrographs shown in Figs 2 and 3.

of ASCII-character stimuli, were presented for five blocks in each test session; each block consisted of one trial with each problem, presented in random order. Thus, each daily session consisted of 40 trials. The inter-trial interval was 10 s. The same eight problems were presented, day after day, until the monkey achieved a criterion of 90% correct or better on each of 2 consecutive days. As in discrimination learning set, there was no visual feedback for correct responses and no correction for errors. After reaching criterion on the set of eight problems, the reward contingencies for each problem were reversed. That is, each previously correct stimulus became incorrect, and vice versa, and the monkey relearned the set of eight problems to the same criterion as before.



Delayed matching-to-sample

On each trial, a sample stimulus appeared in the centre of the screen, and the monkey was rewarded for touching it. After a specified delay interval, two stimuli, the sample and a novel stimulus, appeared, one on the left side of the screen and one on the right. The monkey was rewarded for touching the stimulus that matched the sample; no reward was delivered for touching the other stimulus. The location of the correct stimulus, left or right, was assigned randomly across trials.

The monkeys were trained on this task using a 2-s delay between sample presentation and choice test, and an inter-trial interval of 10 s. One hundred problems were given in each session. After reaching a criterion of 90% correct for 2 consecutive days (i.e. 180 correct responses in 200 trials), the monkeys were given a delayed matching-

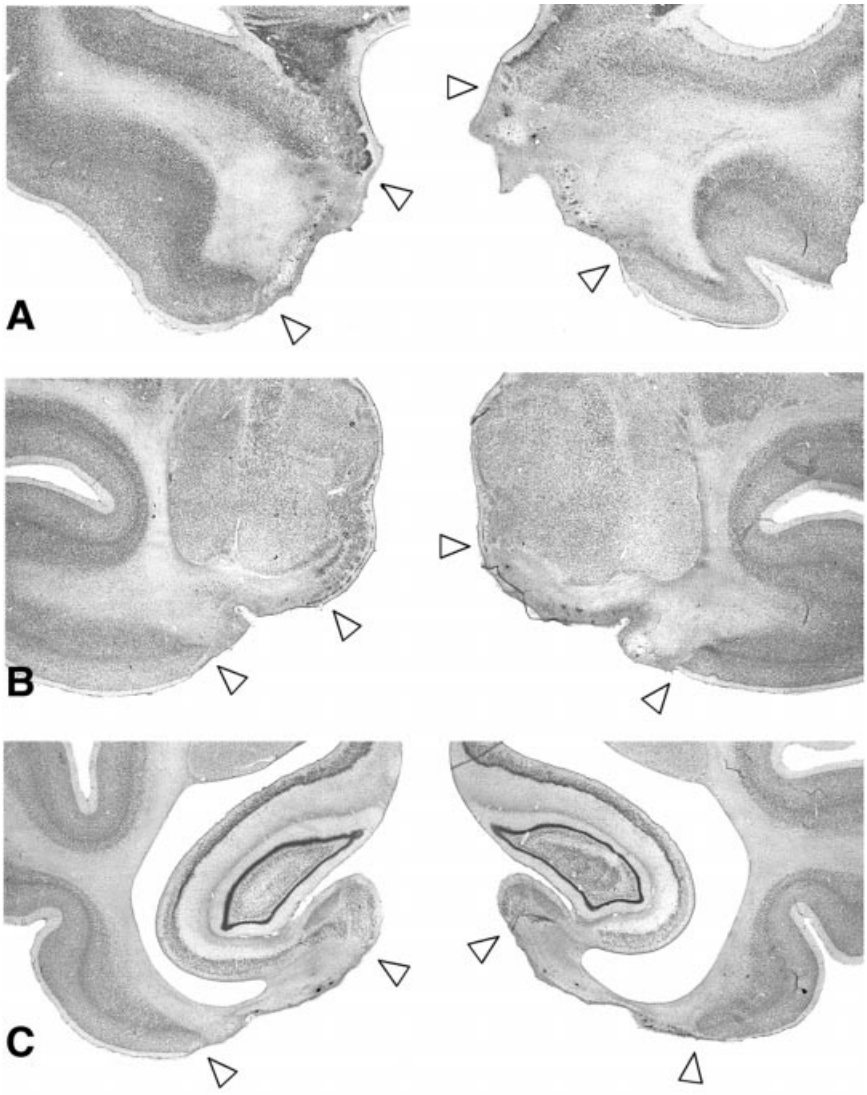


FIG. 2. Photomicrographs of the neurotoxic rhinal cortex lesion in case Rh3. Nissl-stained coronal sections in (A, B and C) show the left and right temporal lobes at approximately 21, 16 and 12 mm anterior to the interaural plane, respectively. Arrowheads mark the medial and lateral edges of the lesion at each level. The rhinal cortex is largely devoid of neurons bilaterally, with relative preservation of non-neuronal elements. Compare and contrast with Fig. 1.

TABLE 1. Percentage damage to entorhinal and perirhinal cortex, as well as the two areas combined ('rhinal') in the four subjects

	Entorhinal damage (%)			Perirhinal damage (%)			Rhinal damage (%)		
Case	L	R	Total	L	R	Total	L	R	Total
Rh1	68.8	59.6	64.2	74.2	78.7	76.5	71.5	69.2	70.3
Rh2	63.1	66.3	64.7	91.1	75.2	83.1	77.0	70.7	73.9
Rh3	76.2	87.8	82.0	86.0	93.4	89.7	81.1	90.6	85.8
Rh4	75.9	71.3	73.6	90.8	79.7	85.3	83.3	75.5	79.4

to-sample (DMS) performance test, in which the delay between sample presentation and choice was 2, 5, 10, 15 or 30 s. One hundred problems were given in each session, and each of the five delays appeared once within each block of five trials. The inter-trial interval was 15 s. Monkeys were required to complete 10 sessions of the DMS performance test.

Preoperative performance test

After completing training on the visual learning tasks, each monkey was retested on all the tasks immediately before surgery, to provide preoperative baseline scores. The tasks were administered in the following order: object discrimination learning set (10 sessions, the last five of which served as the preoperative baseline); single-pair discrimination learning and reversal (two sessions, each preceded by two sessions of discrimination learning without reversal); concurrent object discrimination (to criterion); reversals of the concurrent discriminations (to criterion); DMS at 2 s delay (to criterion); DMS performance test with mixed delays (five sessions). All testing was conducted in the same manner as during initial learning, except that the number of sessions given on each task was fewer.

Postoperative testing

Postoperative testing began 2–5.5 weeks after surgery. Each monkey was first given the second shaping programme that had been used in pretraining. The monkeys were required to complete two sessions of this task before proceeding to a postoperative

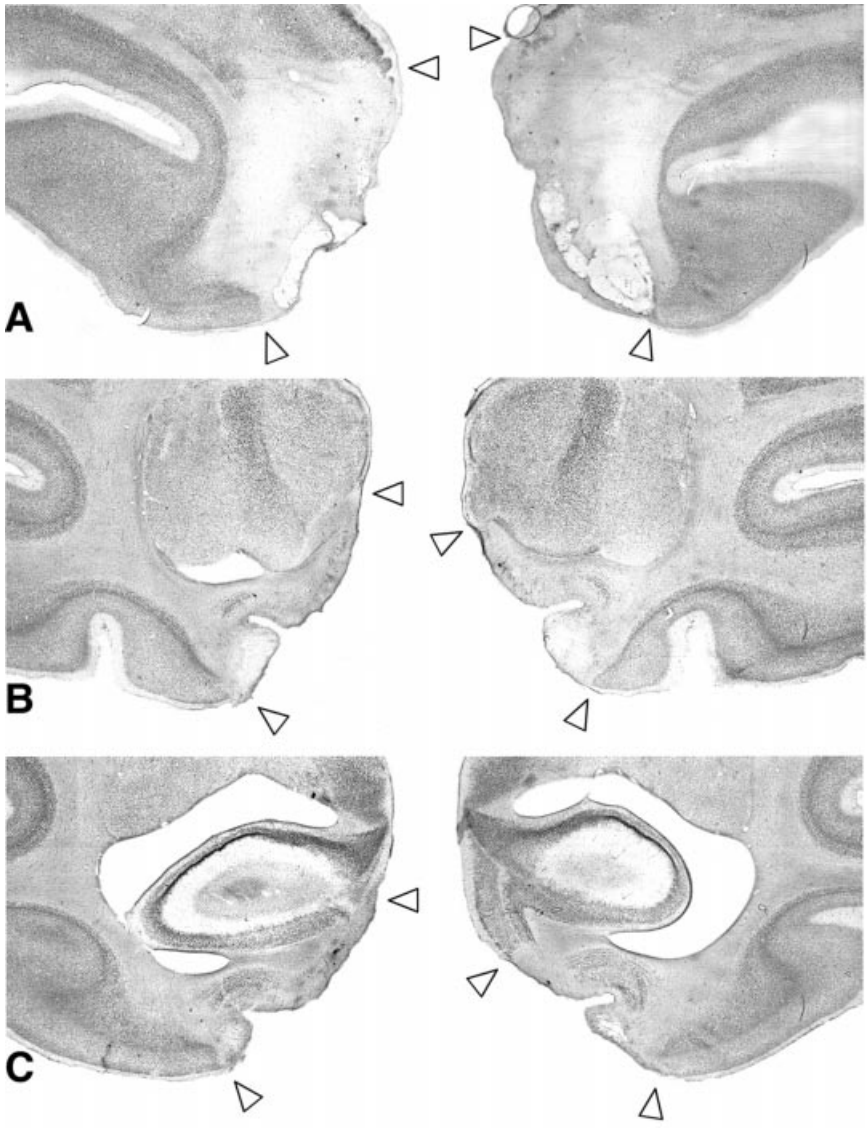


FIG. 3. Photomicrograph of the neurotoxic rhinal cortex lesion in case Rh1. Nissl-stained coronal sections in (A, B and C) show the left and right temporal lobes at approximately 20, 16 and 13 mm anterior to the interaural plane, respectively. Arrowheads mark the medial and lateral edges of the lesion at each level. The rhinal cortex is largely devoid of neurons, though there is sparing of cells in the fundus of the rhinal sulcus, bilaterally (B and C). Non-neuronal elements are relatively well preserved. Compare and contrast with Fig. 1.

performance test on the battery of visual learning tasks, which was administered in the same way it had been immediately prior to surgery. For each task, performance scores on pre- and postoperative performance tests were compared to assess the effects of the neurotoxic rhinal cortex lesions.

This within-subjects design was employed to increase power and facilitate comparison with previous studies of discrimination learning set performance, for which the preoperative performance of each monkey served as its own control (Gaffan & Murray, 1990; Gaffan *et al*., 1993). Although it might be argued that any behavioural deficits observed following the neurotoxic rhinal cortex lesions cannot be interpreted unambiguously in the absence of a corresponding surgical control group, general surgical trauma associated with intracerebral injections of ibotenic acid is unlikely to account for behavioural deficits in the present study. This is because injections of this neurotoxin into other brain structures are without effect on discrimination learning (Málková *et al*., 1997) or visual recognition memory (Murray & Mishkin, 1998). Hence, it seems likely that any behavioural deficits observed following injections of this toxin into the rhinal cortex can be attributed to damage to the cell bodies within the rhinal cortex, rather than nonspecific effects of the surgical procedure.

Surgery

At the time of surgery, anaesthesia was induced with ketamine hydrochloride (10 mg/kg, i.m.) and maintained with isoflurane (1.0–2.0%, to effect). The animals received isotonic fluids via an intravenous drip. Aseptic procedures were employed. Heart rate, respiration rate, blood pressure, expired CO_2 and body temperature were monitored throughout the procedure. A large bone flap was turned over the lateral surface of each hemisphere, and a dural flap was cut to permit access to the ventral surface of the temporal lobe. Between 33 and 50 injections of 1.0 μL ibotenic acid (10 mg/mL, Biosearch Technologies, Novato, CA, USA) were made in the rhinal cortex of each hemisphere via the 30-gauge needle of a Hamilton syringe. Under visual guidance, using the rhinal sulcus as a landmark, the syringe needle was inserted directly into the surface of the rhinal cortex. Each injection was made within approximately 1–2 s, and the needle was held in place for several seconds after the injection. The injections were made rapidly to avoid bruising of the temporal lobe, which was gently retracted to visualize the entire length of the rhinal sulcus. After the injections were completed, the scalp was closed in anatomical layers. All monkeys received a treatment regimen

consisting of dexamethasone sodium phosphate (0.4 mg/kg) and Cefazolin antibiotic (15 mg/kg) for 1 day before surgery and 1 week after surgery to reduce swelling and to prevent infection, respectively. They also received Banamine (flunixin meglumine, 5 mg) for 3 days following surgery, as an analgesic.

Histology

At the completion of the experiment, the monkeys were restrained with ketamine, given a lethal dose of sodium pentobarbital (100 mg/kg, intrahepatic) and transcardially perfused with 0.9% saline followed by a solution of 10% buffered formalin. The brains were removed from the cranium, photographed, postfixed in formalin and cryoprotected in glycerol solutions. Tissue was sectioned at 50 μm on a sliding microtome in the coronal plane. Every fifth section was mounted on gelatine-coated slides, defatted, stained with thionin and coverslipped.

The lesions were generally as intended. Each monkey sustained massive cell loss in the entorhinal and perirhinal cortex bilaterally. Damage to neighbouring regions was slight. In addition, preservation of non-neuronal elements was good; vacuolization and general tissue disruption in the area of the lesions was relatively infrequent. The location and extent of the lesions is illustrated in Fig. 1, and summarized in Table 1. Photomicrographs of Nissl-stained sections through the lesion in two cases are shown in Figs 2 and 3. Damage to the entorhinal cortex ranged from 64.2 to 82.0% bilaterally, and damage to the perirhinal cortex ranged from 76.5 to 89.7%. There was typically some sparing of the medial entorhinal cortex, especially at caudal levels, as well as some sparing of cells in the fundus of the rhinal sulcus at intermediate levels of the lesion. With regard to unintended damage, there was some damage to temporal polar area TG and to the piriform cortex in all four cases. For both of these areas, the extent of damage was much less than that typically seen following aspiration lesions of the rhinal cortex (compared with, for example, cases Rh1–Rh7 in Meunier *et al.*, 1993). In addition, all four cases sustained slight damage to area TE, which consisted of less than 3% of the volume of this area. In two cases (Rh3 and Rh4), this damage appeared to be due to infarcts that occurred during surgery, presumably as a consequence of the retraction of the temporal lobe. These small areas of unilateral cell loss were not contiguous with the rhinal cortex lesion and did not resemble damage associated with ibotenic acid injections. All four cases also sustained slight damage to the ventral amygdala at rostral levels; this ranged from 2.1 to 9.8% and was greatest in case Rh1. Finally, cases Rh3 and Rh4 also sustained slight damage to hippocampal cell field CA1, for 3 mm on the left in case Rh3 and 2 mm on the right in case Rh4, and cases Rh2 and Rh4 sustained slight damage to parahippocampal cortical areas TH and TF on the left, for 1 mm in case Rh2 and 2 mm in case Rh4.

Results

Discrimination learning set

The mean percentage correct on trials 2–20 of each of the 25 problems learned during the final five sessions of the pre- and postoperative performance tests was calculated and used as the dependent measure (Fig. 4). Each monkey performed more poorly postoperatively compared with its own preoperative performance; paired $t_3 = 3.83$, $P = 0.031$.

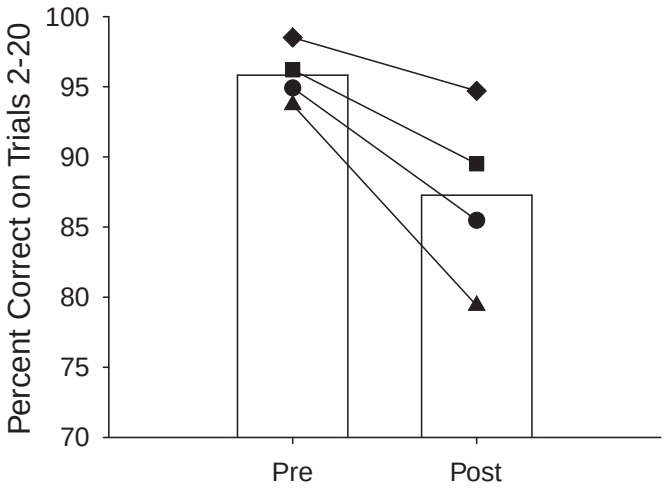


FIG. 4. Discrimination learning set performance. Data illustrated are the mean percentage correct across trials 2–20 of 25 new problems learned in the pre- and postoperative performance tests. As a group, the monkeys are significantly impaired postoperatively on learning of new discrimination problems. Bars indicate the group means; symbols indicate the scores of individual monkeys: Rh1, circle; Rh2, square; Rh3, triangle; Rh4, diamond.

Single-pair discrimination learning and reversals

The mean errors to reach the initial learning and reversal criteria for each discrimination across the four problems in the pre- and postoperative performance tests were calculated and used as the dependent measure (Fig. 5). The monkeys found this task extremely easy, evinced by the low number of errors to criterion on learning and reversal of each problem. Although numerically more errors were made on reversals than during initial learning both preoperatively and postoperatively, this effect did not reach significance, $F_{1,3} = 3.71$, $P = 0.15$; similarly, there was no main effect of lesion, $F_{1,3} = 0.85$, $P = 0.42$, or lesion by learning–reversal interaction, $F_{1,3} = 3.61$, $P = 0.15$. Comparison of the errors to criterion on reversal of the first problem encountered postoperatively, or of the mean errors to criterion on the two reversals encountered in the first postoperative session, to preoperative performance also revealed no significant impairment, $t_3 < 0.97$, $P > 0.41$.

Concurrent discrimination learning and reversal

The number of errors accrued in attaining criterion (including errors committed during the criterion run) for learning and reversal of the eight concurrent discrimination problems in the pre- and post-operative performance tests was used as the dependent measure (Fig. 6). In this task, the reversals were more challenging than the initial learning, $F_{1,3} = 41.8$, $P = 0.008$; however, the monkeys were not impaired overall postoperatively, nor were they differentially affected on the reversals postoperatively, $F_{1,3} < 2.43$, $P > 0.22$. Identical results were obtained with an analysis of the number of sessions to criterion (data not shown).

Delayed matching-to-sample

Monkeys were not reliably impaired in reacquiring the DMS task postoperatively; mean sessions to criterion (including the criterion run) in the preoperative and postoperative phases were 2.25 (range 2–3) and 4.75 (range 2–10), respectively; paired $t_3 = 1.61$, $P = 0.21$. The performance test scores, taken as the mean percentage correct at each of the five delays, served as the dependent measures (Fig. 7; Table 2). Monkeys were significantly impaired on DMS postoperatively and this

impairment appeared to be exacerbated as the delay interval between the sample presentation and choice test increased. Analysis of variance (ANOVA) using data from all five delay conditions revealed a main effect of lesion, $F_{1,3} = 21.59$, $P = 0.019$; a main effect of delay, $F_{4,12} = 18.11$, $P < 0.0005$; and a lesion-by-delay interaction, $F_{4,12} = 16.64$, $P < 0.0005$. The presence of a significant lesion-by-delay interaction suggests that the impairment on this task is due to a deficit in recognition memory, as postoperative performance is more severely affected at the longer delays.

The significant lesion-by-delay interaction could be an artefact of the inclusion of the data for the 2-s delay condition, on which the monkeys had received extended practice during reacquisition of DMS before the variable-delay performance test. However, repeating this analysis after exclusion of these data revealed precisely the same pattern of effects: main effect of lesion, $F_{1,3} = 30.76$, $P = 0.012$; main effect of delay, $F_{3,9} = 16.85$, $P < 0.0005$; and a lesion-by-delay interaction, $F_{3,9} = 13.43$, $P = 0.001$.

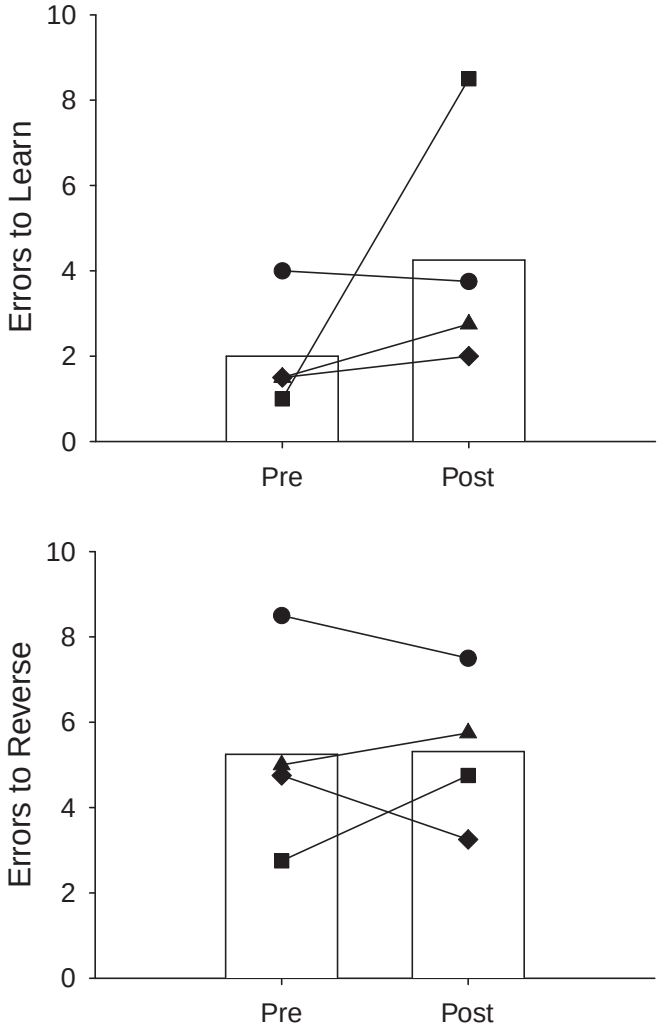


FIG. 5. Single-pair discrimination reversal performance. Data illustrated are the mean number of errors to criterion to learn (top panel) or reverse (bottom panel) single discriminations, across four new problems learned in the pre- and postoperative performance tests. There is no reliable postoperative impairment in new learning or reversal of these problems. Bars indicate the group means and symbols indicate the scores of individual monkeys: Rh1, circle; Rh2, square; Rh3, triangle; Rh4, diamond.

Discussion

Aspiration vs. neurotoxic lesions of the rhinal cortex

There was a significant correspondence between the effects of neurotoxic rhinal cortex lesions observed in the present study with results previously described after aspiration lesions of this structure. Specifically, monkeys with neurotoxic rhinal cortex lesions were impaired on single-pair visual discrimination learning set and visual recognition memory, both of which are impaired by aspiration lesions of the rhinal cortex (Meunier *et al*., 1993; Eacott *et al*., 1994; Baxter *et al*., 1999), but not on concurrent discrimination learning with a set of eight problems, which is intact after aspiration lesions of the perirhinal cortex (Buffalo *et al*., 1999). This correspondence validates the interpretation of previous lesion studies that used the aspiration technique to damage the rhinal cortex, which attributed the behavioural deficits to damage to the cell bodies within the rhinal cortex. Furthermore, this suggests that behavioural impairments on these tasks associated with aspiration lesions of the rhinal cortex are not caused by disruption of fibres of passage, for instance those fibres travelling between the hippocampus and amygdala (see figs 6 and 10 of Saunders *et al*., 1988), between the temporal cortex and hippocampal formation (Leonard *et al*., 1995; Rockland & Van Hoesen, 1999) or between the inferior parietal lobule and anterior presubiculum (e.g. Ding *et al*., 2000; G. Van Hoesen, personal communication).

Our monkeys with neurotoxic rhinal cortex lesions were not reliably impaired on reversal learning, either of rapidly learned single discrimination problems or of eight concurrent discriminations learned gradually across sessions, although two of the four monkeys demonstrated an increase in postoperative errors to reverse concurrent discriminations. By contrast, monkeys with aspiration lesions of the rhinal cortex have been reported to be significantly impaired on reversals of single-object discrimination problems (Murray *et al*., 1998a). There are at least three possible explanations of this apparent discrepancy. First, the lesions in the earlier study could have been

TABLE 2. Pre-and postoperative scores on delayed matching-to-sample for each delay condition and for each monkey

	Preoperative score (% correct)					Postoperative score (% correct)					Mean score (% correct)	
Case	2 s	5 s	10 s	15 s	30 s	2 s	5 s	10 s	15 s	30 s	Pre	Post
Rh1	98	100	99	98	89	84	92	77	69	59	96.5	74.3
Rh2	99	100	98	96	87	100	98	90	75	62	95.3	81.3
Rh3	100	98	94	94	93	96	91	78	85	73	94.8	81.8
Rh4	99	99	99	100	97	98	99	95	85	77	98.8	89.0

Scores shown as percentage correct for each delay condition (2 s-30 s), as well as pre- and postoperative performance test scores (mean), excluding the training (2 s) delay.

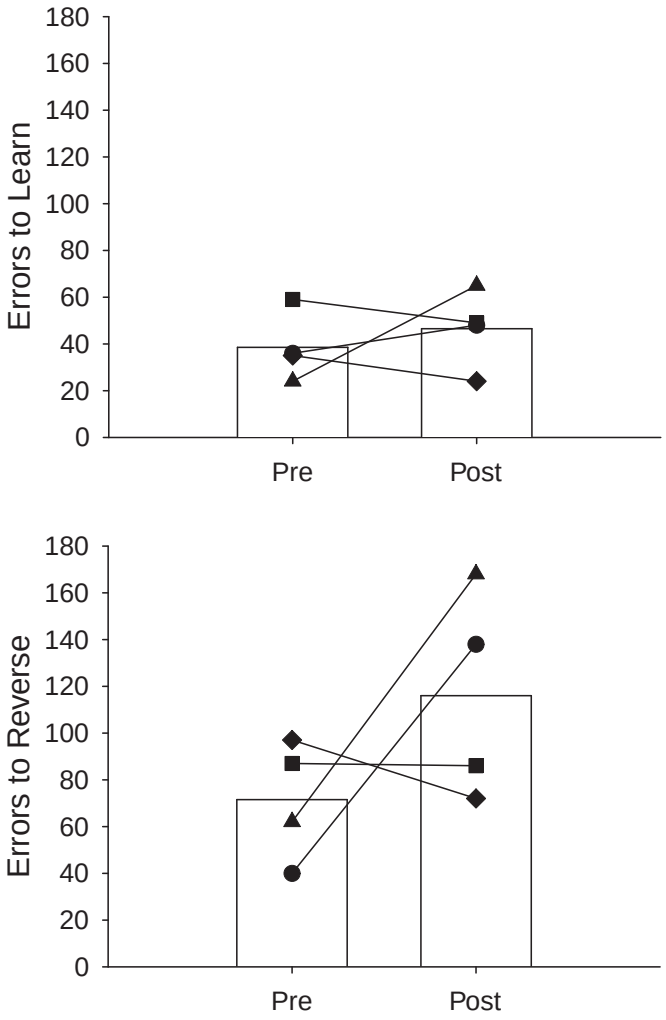


FIG. 6. Concurrent discrimination learning and reversal. Data illustrated are the number of errors to criterion for learning (top panel) or reversal (bottom panel) of eight visual discrimination problems, presented concurrently within each session. Reversal of concurrent discriminations is significantly more difficult than initial learning, but there is no reliable effect of lesion on either learning or reversal of these problems. Bars indicate the group means and symbols indicate the scores of individual monkeys: Rh1, circle; Rh2, square; Rh3, triangle; Rh4, diamond.

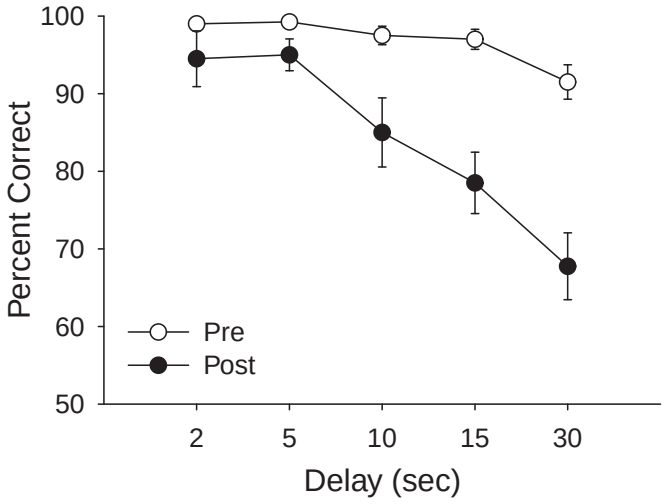


FIG. 7. Delayed matching-to-sample performance test. Data illustrated are the percentage correct responses obtained in the five sessions of testing in the preoperative (open symbols) and postoperative (closed symbols) performance tests; error bars indicate the standard error of the mean. Monkeys are significantly impaired overall after neurotoxic rhinal cortex lesions and are differentially impaired on the longer delays.

more complete than the lesions in the present study; this can be easily discounted because the volume of rhinal cortex damage in the present study is actually larger than that in the previous study and the extent of damage to area TE is also similar in the two studies (Murray *et al*., 1998a). Second, it is possible that the earlier-reported deficit in reversal learning was due to the interruption by aspiration lesions of fibres of passage through the rhinal cortex, *en route* to some other structure, rather than damage to the cell bodies of the rhinal cortex itself. Third, and most likely, the apparent discrepancy could be due to the different training procedures used in the two studies. Whereas monkeys in the present study received extensive preoperative experience with single-pair and concurrent reversal problems before the neurotoxic rhinal cortex lesions were produced, monkeys in the prior study (Murray *et al*., 1998a) encountered reversal problems for the first time postoperatively. We hypothesized in the previous study that the reversal impairment in monkeys with rhinal cortex damage, which resembled that of monkeys with orbital prefrontal cortex damage, might reflect the preferential connectivity of the rhinal cortex with the orbital prefrontal cortex (Murray *et al*., 1998a). It has been noted that monkeys with prefrontal cortex damage display reversal learning deficits, as well as other behavioural deficits related to shifting of attention to different stimulus dimensions, only on the first instance in which these behavioural problems are encountered (Dias *et al*., 1997). Hence, perhaps the extensive preoperative experience with reversals reduced the effect of the lesion on reversal learning in the present experiment, because reversal problems (both single-pair and concurrent) had been encountered many times before the rhinal cortex lesions were sustained.

Rhinal cortical contributions to discrimination learning

Monkeys with damage limited to the rhinal cortex were mildly impaired on the discrimination learning set task used in the present study, which uses primary reinforcement to signal the correct stimulus (one of two ASCII-character stimuli). Monkeys with aspiration lesions of the rhinal cortex display a similar mild impairment on visual discrimination learning for the identical type of stimuli, when the correct stimulus is signalled by an auditory secondary reinforcer and must be chosen on four consecutive trials before primary reinforcement is delivered (Baxter *et al*., 1999). Hence, the mild impairment produced by rhinal cortex damage in learning discrimination problems of this type is not limited to situations in which the reinforcer is a secondary reinforcer of a different sensory modality.

Discrimination learning for primary reinforcement, like visual discrimination learning for an auditory secondary reinforcer, is severely impaired by aspiration lesions of the amygdala (Gaffan & Harrison, 1987; Gaffan & Murray, 1990), which interrupt projections of both rhinal cortex and area TE to prefrontal and thalamic targets (Baxter *et al*., 1998; Goulet *et al*., 1998). We have argued elsewhere that effects of amygdala aspiration lesions on discrimination learning cannot be accounted for entirely by damage to the rhinal cortex (whether direct or indirect), and likely reflects the disconnection of an inferior temporal-thalamic-prefrontal network (Baxter & Murray, 2000). This conclusion is based primarily on the observation that whereas aspiration lesions limited to the rhinal cortex produce only a

mild deficit in visual discrimination learning for an auditory secondary reinforcer, aspiration lesions of rhinal cortex plus area TE produce a more severe deficit, similar in magnitude to that resulting from aspiration lesions of the amygdala (Baxter *et al*., 1999). The results of the present study are congruent with this conclusion, in that the deficit produced by neurotoxic rhinal cortex lesions on discrimination learning for primary reinforcement (a mean difference in pre- and postoperative performance of 8.56% correct) is also milder than that produced by aspiration lesions of the amygdala on this same task (a difference in pre- and postoperative performance of 16.3%; Gaffan & Murray, 1990).

We found in the present study that monkeys with rhinal cortex lesions were impaired on single-pair discrimination problems (i.e. on discrimination learning set), but not in an eight-pair concurrent discrimination task with similar types of stimuli, a finding analogous to that reported by Buffalo *et al*. (1999). Such a finding is in apparent conflict with other reports of discrimination learning impairments following perirhinal cortex lesions, in which acquisition of small sets of concurrent discrimination problems is unimpaired, but acquisition of large sets of concurrent discrimination problems is impaired (Buckley & Gaffan, 1997). Effects of rhinal cortex damage on visual discrimination learning are inconsistent across different studies, and likely depend on the qualities of objects to be discriminated (two-dimensional vs. three-dimensional; real objects vs. computer-generated graphic stimuli, complex shapes vs. ASCII characters, etc.; Buckley & Gaffan, 1997, 1998a; Thornton *et al*., 1997, 1998; Murray *et al*., 1998a; Baxter *et al*., 1999; Bussey *et al*., 1999; for review see Baxter, 2001), as well as the size of the lesions. In our previous study of visual discrimination learning for an auditory secondary reinforcer in monkeys with rhinal cortex lesions (Baxter *et al*., 1999), we suggested that the mild impairment caused by rhinal cortex lesions was due to the requirement of discriminating many stimuli with similar physical characteristics, thereby taxing visual perceptual abilities, to which the rhinal cortex contributes (Buckley & Gaffan, 1997; Murray *et al*., 1998b; Murray & Bussey, 1999).

The single-pair impairment observed in the present study may reflect a particular sensitivity of the discrimination learning set procedure to rhinal cortex damage, in which single discrimination problems are learned rapidly and the monkeys have received extensive preoperative training in this procedure. It is also possible that the rate of learning in the eight-pair concurrent task masked a postoperative impairment; it is interesting to note that difference scores for concurrent discrimination learning and reversal (i.e. difference in the number of pre- and postoperative errors to criterion in each condition) are highly correlated with difference scores for discrimination learning set ($r > 0.91$). Thus, these tasks may all be indexing a common and relatively mild impairment in visual perceptual abilities. Consistent with this idea, Easton & Gaffan (2000) have recently reported a significant impairment in 10-pair concurrent learning after perirhinal cortex lesions in monkeys. In the end, the appearance of an impairment after rhinal cortex damage may depend more on the nature of the stimulus material than on the number of stimuli to be discriminated at any one time (Bussey *et al*., 1999; Murray & Bussey, 1999).

Recognition vs. discrimination in rhinal cortex

Because the monkeys in the present study were tested on multiple behavioural tasks, we had the opportunity to examine the relationship between postoperative impairments in object discrimination learning and in visual recognition memory. This is germane to the issue of whether a unitary perceptual-mnemonic function might be ascribed to the rhinal cortex (e.g. Murray & Bussey, 1999). The specific question was whether postoperative deficits in discrimination learning were related to deficits in recognition memory, assessed using the DMS task. Effects of rhinal cortex lesions on discrimination learning, measured as a difference score between pre- and postoperative performance, were not reliably correlated with deficits on the DMS performance test measured by a similar difference score, excluding data obtained in the training (2 s) delay condition ($r = 0.305$). Hence, the magnitude of discrimination learning impairment did not predict the magnitude of recognition memory impairment. This suggests that impairments in recognition memory following rhinal cortex damage do not simply reflect a deficit in object identification or discrimination ability. The possible anatomical or physiological substrates of such a dissociation remain unclear, but the absence of an association between object discrimination impairment and object recognition impairment suggests that these memory functions are mediated by separate mechanisms within the rhinal cortex. This conclusion is bolstered by the observation that recognition memory deficits persist in monkeys with perirhinal cortex lesions, even when the perceptual identification of the to-be-remembered stimuli is equated with that of controls (Hampton & Murray, 2000).

Acknowledgements

We thank Tinera Fobbs, Wendy Hadfield, Alicia Izquierdo and John Sewell for technical assistance. We especially thank Richard Saunders for performing the surgery. This research was supported by the National Institute of Mental Health Intramural Research Program.

Abbreviations

ANOVA, analysis of variance; DMS, delayed matching-to-sample; i.m., intramuscularly.

References

Baxter, M.G., Hadfield, W.S. & Murray, E.A. (1999) Rhinal cortex lesions produce mild deficits in visual discrimination learning for an auditory secondary reinforcer in rhesus monkeys. *Behav. Neurosci.*, **113**, 243–252.

Baxter, M.G. & Murray, E.A. (2000) Reinterpreting the behavioral effects of amygdala lesions in nonhuman primates. In Aggleton, J.P. (ed.), *The Amygdala: a Functional Analysis*. Oxford University Press, Oxford, pp. 545–568.

Baxter, M.G., Saunders, R.C. & Murray, E.A. (1998) Aspiration lesions of the amygdala interrupt connections between prefrontal cortex and temporal cortex in rhesus monkeys. *Soc. Neurosci. Abstr.*, **24**, 1905.

Baxter, M.G. (2001) Differentiating the contribution of the primate rhinal cortex to learning and memory. In Parker, A., Wilding E. & Bussey, T. (eds.). *The Cognitive Neuroscience of Memory Formation.* Psychology Press, East Sussex, in press.

Buckley, M.J. & Gaffan, D. (1997) Impairment of visual object-discrimination learning after perirhinal cortex ablation. *Behav. Neurosci.*, **111**, 467–475.

Buckley, M.J. & Gaffan, D. (1998a) Learning and transfer of object–reward associations and the role of the perirhinal cortex. *Behav. Neurosci.*, **112**, 15–23.

Buckley, M.J. & Gaffan, D. (1998b) Perirhinal cortex ablation impairs visual object identification. *J. Neurosci.*, **18**, 2268–2275.

Buffalo, E.A., Ramus, S.J., Clark, R.E., Teng, E., Squire, L.R. & Zola, S.M. (1999) Dissociation between the effects of damage to perirhinal cortex and area TE. *Learn. Mem.*, **6**, 572–599.

Bussey, T.J., Saksida, L.M. & Murray, E.A. (1999) Overgeneralization in monkeys with perirhinal cortex lesions. *Soc. Neurosci. Abstr.*, **25**, 789.

Dias, R., Robbins, T.W. & Roberts, A.C. (1997) Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: restriction to novel situations and independence from 'on-line' processing. *J. Neurosci.*, **17**, 9285–9297.

Ding, S.L., Van Hoesen, G. & Rockland, K.S. (2000) Inferior parietal lobule

projections to the presubiculum and neighboring ventromedial temporal cortical areas. *J. Comp. Neurol.*, **425**, 510–530.
Dunn, L.T. & Everitt, B.J. (1988) Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. *Behav. Neurosci.*, **102**, 3–23.
Eacott, M.J., Gaffan, D. & Murray, E.A. (1994) Preserved recognition memory for small sets, and impaired stimulus identification for large sets, following rhinal cortex ablations in monkeys. *Eur. J. Neurosci.*, **6**, 1466–1478.
Easton, A. & Gaffan, D. (2000) Comparison of perirhinal cortex ablation and crossed unilateral lesions of the medial forebrain bundle from the inferior temporal cortex in the rhesus monkey: Effects on learning and retrieval. *Behav. Neurosci.*, **114**, 1041–1057.
Friedman, D.P., Murray, E.A., O'Neill, J.B. & Mishkin, M. (1986) Cortical connections of the somatosensory fields of the lateral sulcus of macaques: evidence for a corticolimbic pathway for touch. *J. Comp. Neurol.*, **252**, 323–347.
Gaffan, D. & Harrison, S. (1987) Amygdalectomy and disconnection in visual learning for auditory secondary reinforcement by monkeys. *J. Neurosci.*, **7**, 2285–2292.
Gaffan, D. & Murray, E.A. (1990) Amygdalar interaction with the mediodorsal nucleus of the thalamus and the ventromedial prefrontal cortex in stimulus-reward associative learning in the monkey. *J. Neurosci.*, **10**, 3479–3493.
Gaffan, D., Murray, E.A. & Fabre-Thorpe, M. (1993) Interaction of the amygdala with the frontal lobe in reward memory. *Eur. J. Neurosci.*, **5**, 968–975.
Goulet, S., Doré, F.Y. & Murray, E.A. (1998) Aspiration lesions of the amygdala disrupt the rhinal corticothalamic projection system in rhesus monkeys. *Exp. Brain Res.*, **119**, 131–140.
Goulet, S. & Murray, E.A., (2001) Neural substrates of crossmodal association memory in monkeys: the amygdala versus the anterior rhinal cortex. *Behav. Neurosci.*, in press.
Hampton, R.R. & Murray, E.A. (2000) Deficits following perirhinal cortex lesions: Representation or retention? *Soc. Neurosci. Abstr.*, **26**, 545.
Leonard, B.W., Amaral, D.G., Squire, L.R. & Zola-Morgan, S. (1995) Transient memory impairment in monkeys with bilateral lesions of the entorhinal cortex. *J. Neurosci.*, **15**, 5637–5659.
Málková, L., Gaffan, D. & Murray, E.A. (1997) Excitotoxic lesions of the amygdala fail to produce impairments in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. *J. Neurosci.*, **17**, 6011–6020.
Meunier, M., Bachevalier, J., Mishkin, M. & Murray, E.A. (1993) Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J. Neurosci.*, **13**, 5418–5432.
Murray, E.A. (1992) Medial temporal lobe structures contributing to recognition memory: The amygdaloid complex versus the rhinal cortex. In Aggleton, J.P. (ed.) *The Amygdala Neurobiological Aspects of Emotion, Memory and Mental Dysfunction*. New York, Wiley-Liss, pp. 453–470.
Murray, E.A. (2000) Memory for objects in nonhuman primates. In Gazzaniga, M.S. (ed.), *The New Cognitive Neurosciences*. MIT Press, Cambridge, MA, pp. 753–763.
Murray, E.A., Baxter, M.G. & Gaffan, D. (1998a) Monkeys with rhinal cortex damage or neurotoxic hippocampal lesions are impaired on spatial scene learning and object reversals. *Behav. Neurosci.*, **112**, 1291–1303.
Murray, E.A. & Bussey, T.J. (1999) Perceptual-mnemonic functions of the perirhinal cortex. *Trends Cognit. Sci.*, **3**, 142–151.
Murray, E.A., Gaffan, D. & Mishkin, M. (1993) Neural substrates of visual stimulus–stimulus association in rhesus monkeys. *J. Neurosci.*, **13**, 4549–4561.
Murray, E.A., Málková, L. & Goulet, S. (1998b) Crossmodal associations, intramodal associations, and object identification in macaque monkeys. In Milner, A.D. (ed.), *Comparative Neuropsychology*. Oxford University Press, Oxford, pp. 51–67.
Murray, E.A. & Mishkin, M. (1998) Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *J. Neurosci.*, **18**, 6568–6582.
Parker, A. & Gaffan, D. (1998) Lesions of the primate rhinal cortex cause deficits in flavour-visual associative memory. *Behav. Brain Res.*, **93**, 99–105.
Rockland, K.S. & Van Hoesen, G.W. (1999) Some temporal and parietal cortical connections converge in CA1 of the primate hippocampus. *Cereb. Cortex*, **9**, 232–237.
Saunders, R.C., Rosene, D.L. & Van Hoesen, G.W. (1988) Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. Reciprocal and non-reciprocal connections. *J. Comp. Neurol.*, **271**, 185–207.
Suzuki, W.A. (1996) Neuroanatomy of the monkey entorhinal, perirhinal and parahippocampal cortices: Organization of cortical inputs and interconnections with amygdala and striatum. *Sem. Neurosci.*, **8**, 3–12.
Suzuki, W.A. & Amaral, D.G. (1994) Perirhinal and parahippocampal cortices of the macaque monkey: cortical afferents. *J. Comp. Neurol.*, **350**, 497–533.
Thornton, J.A., Málková, L. & Murray, E.A. (1998) Rhinal cortex ablations fail to disrupt reinforcer devaluation effects in rhesus monkeys (*Macaca mulatta*). *Behav. Neurosci.*, **112**, 1020–1025.
Thornton, J.A., Rothblat, L.A. & Murray, E.A. (1997) Rhinal cortex removal produces amnesia for preoperatively learned discrimination problems but fails to disrupt postoperative acquisition and retention in rhesus monkeys. *J. Neurosci.*, **17**, 8536–8549.