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RESEARCH ARTICLE

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Spontaneous object recognition and object location memory in rats: the effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix

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Abstract The first experiment assessed the effects of neurotoxic lesions in either the anterior cingulate cortex (ACc) or the retrosplenial cortex (RSc) on a test of object recognition. Neither lesion affected performance on this task, which takes advantage of the rat's normal preference to spend more time investigating novel rather than familiar stimuli. In response to this negative result, a second experiment assessed the effects of much more extensive cingulate lesions (Cg) on both object recognition and object location memory. The latter task also used a preference measure, but in this case it concerned preference for a novel location. For comparison purposes this second study included groups of rats with lesions in closely allied regions: the fornix (Fx), the cingulum bundle (CB) and the medial prefrontal cortex (Pfc). Comparisons with sham-operated control rats showed that none of the four groups (Cg, Fx, CB, Pfc) was impaired on the object recognition task, adding further weight to the view that these structures are not necessary for assessing stimulus familiarity. The Fx and Cg groups were, however, impaired on the object location task, suggesting that these regions are necessary for remembering other attributes of a stimulus (spatial location).

Key words Fornix · Cingulate cortex · Prefrontal cortex · Cingulum bundle · Hippocampus · Object recognition · Object location · Memory · Rat

Introduction

It has been demonstrated in a series of studies (Ennaceur and Delacour 1988; Ennaceur and Meliani 1992a,b; Ennaceur and Aggleton 1994; Ennaceur et al. 1996a,b) that spontaneous exploratory activity in the rat can be used to provide a valid measure of memory function. For example, object recognition can be assessed from the preference normal rats display for investigating novel rather than familiar complex objects (Ennaceur and Delacour 1988). This preference is reliably observed for delays of 15 min, but when retention delays are increased to 60 min it becomes less consistent. The preference for novelty is also influenced in a predictable manner by factors such as sample duration and the inclusion of common features in the familiar sample stimulus and the novel alternative (Ennaceur and Delacour 1988; Ennaceur and Aggleton 1994; Ennaceur et al. 1996a,b). Advantages associated with this class of measure include the fact that performance does not depend on the retention of a rule, nor is it influenced by changes in responsivity to reward. Furthermore, because the test uses a forced-choice design it is less likely to be affected by changes in impulsivity or activity. As a consequence such tasks can provide a relatively pure measure of "working memory" (Honig 1978; Olton et al. 1979).

Previous studies using this test of spontaneous object recognition have shown that performance is susceptible to various drug and lesion treatments (Ennaceur and Delacour 1987; Ennaceur et al. 1989; Ennaceur 1991; Mickley et al. 1994; Scali et al. 1994; Cobb et al. 1995; Giovannelli et al. 1995; Hlinak and Krejci 1995; Bartolini et al. 1996). It has, for example, been demonstrated that rats with rhinal damage (Ennaceur et al. 1996a,b), scopolamine-treated rats (Ennaceur and Meliani 1992b), and rats receiving chronic administration of a nitric oxide synthase inhibitor (Cobb et al. 1995) show indifferent exploration between the familiar and the new object. The fact that they fail to discriminate between the objects indicates that the treatments have disrupted the ability of the animal to identify the familiar object. In contrast, other manipulations do not appear to disrupt spontaneous preference. These include transection of the fornix (Ennaceur and Aggleton 1994; Ennaceur et al. 1996a), lesions of the medial septum (Ennaceur and

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Meliani 1992a) and lesions of the anterior thalamic nuclei (Aggleton et al. 1995a). These null results are of interest as all three structures are closely linked with the hippocampus, and it has been proposed from the outcome of primate studies that the hippocampus is importantly involved in recognition (Squire and Zola-Morgan 1991; Squire 1992; Alvarez et al. 1995). Indeed, it has been claimed that tests of spontaneous novelty preference might be especially sensitive to hippocampal damage (Alvarez et al. 1995). These proposals remain controversial, as the critical involvement of the hippocampus has been challenged (Mishkin and Murray 1994; Aggleton and Shaw 1996).

For these reasons we examined spontaneous object recognition following lesions in a number of structures that are closely linked with the hippocampus. The first experiment looked at the effects of selective lesions within the cingulate region. These were targeted at either the anterior cingulate cortex or the retrosplenial cortex (Vogt and Peters 1981). The lesions were made by injecting *N*-methyl-D-aspartic acid (NMDA). This technique was chosen as it spares the underlying cingulum bundle, which might otherwise contribute to any lesion effect (Meunier and Destrade 1988; Aggleton et al. 1995b). The group with retrosplenial lesions was of especial interest in view of the dense interconnections between this region and the hippocampus (Wyss and van Groen 1992), and the associated evidence of a close functional relationship (Sutherland and Hoesing 1993). Further interest in the retrosplenial cortex arises from a report that damage in this area might be sufficient to induce anterograde amnesia (Valenstein et al. 1987). For comparison purposes, a group of rats with fornix lesions and a group of surgical control animals were included in the experiment.

This study was followed by a second experiment in which the neurotoxic anterior cingulate and retrosplenial lesions were combined so as to maximise the likelihood of a cingulate lesion effect on the familiarity test. As previous studies have shown that aspiration lesions of a similar extent of cingulate cortex are sufficient markedly to impair certain spatial working memory tasks that are sensitive to hippocampal damage (Sutherland et al. 1988; Markowska et al. 1989) the rats were tested on a second spontaneous preference task which assessed object location memory. In this task the rats are presented with two identical, familiar objects, one of which is in its previous location while the other is in a new location. Normal rats spontaneously spend more time exploring the object in the novel location (Ennaceur and Meliani 1992a). A group of rats with radiofrequency lesions of the cingulum bundle were also tested on the two preference tasks. The rationale for including this group arises from the fact that this tract contains fibres linking the hippocampus with the cingulate cortex and prefrontal cortex, and from evidence that the effects of conventional cingulate lesions might be exacerbated by inadvertent damage to this tract (Meunier and Destrade 1988; Neave et al. 1994; Aggleton et al. 1995b). A group of rats with fornix lesions was also included, to confirm the sensitivity of this spatial task.

A final group of rats with neurotoxic lesions of the medial prefrontal cortex was included in this second experiment. Interest in the effects of this lesion arose from evidence that extensive damage to the medial and orbital prefrontal cortices can impair recognition judgements in monkeys (Bachevalier and Mishkin 1986). Furthermore, studies with rats have shown that damage to the medial prefrontal cortex can disrupt a variety of spatial working memory tasks (Brito et al. 1982; Brito and Brito 1990; Dunnett 1990; Shaw and Aggleton 1993; Aggleton et al. 1995), although there is uncertainty as to whether the deficits stem from the nature of the stimuli, the involvement of working memory, the particular response demands, or a combination of these factors (Dunnett 1990; de Bruin et al. 1994; Aggleton et al. 1995b). In view of the fact that preferential exploratory tasks offer a relatively pure measure of working memory they may provide a particularly valuable way of assessing the impact of medial prefrontal damage. The animals in both experiments had, prior to the study, been tested on an automated delayed nonmatching-to-position task and were subsequently tested on a T-maze alternation task (Neave et al. 1994; Aggleton et al. 1995b).

General methods

Apparatus

The apparatus consisted of an open box (100×100×50 cm high) made of aluminium with the inside painted matt grey. The floor was covered with woodchip bedding which was moved around between trials/days to stop build-up of odour in certain sites. The objects to be discriminated were available in four copies and made of an inert material such as glass, plastic or metal (Fig. 1). The weight of the objects ensured that they could not be displaced by the rats. To achieve this, some objects were filled with water or sand.

Testing procedure

All animals initially received three sessions of 10 min duration in the empty box to help them habituate to the apparatus and the test room. For this and the test procedure the rats were observed on a screen monitor connected to a video camera suspended above the test arena. In the object recognition task, each rat was first placed in the box and exposed to two identical sample stimuli (A1 and A2) for 3 min. These objects were placed close (approximately 10 cm) to two adjacent corners. The rat was then returned to its cage. During the retention interval, the experimenter removed both objects and replaced one by its identical copy (A3) and the other one by a new object (B). After a delay of 1 min or 15 min the rat was placed back in the box and now exposed to the familiar object (A3, object identical to A1 and A2) and a novel test object (B, new object) for a further 3 min. These were in the same locations as the previous objects. An experimenter, who did not know the group identity of the individual rats, recorded the total time spent exploring the two objects in both the sample and the test periods. Exploration was operationally defined as directly attending to the object with the head no more than 2 cm from the object (Ennaceur and Delacour 1988). New objects were used for every session.

The first phase of the object location test was exactly the same as the recognition test. Thus two identical sample stimuli (A1 and

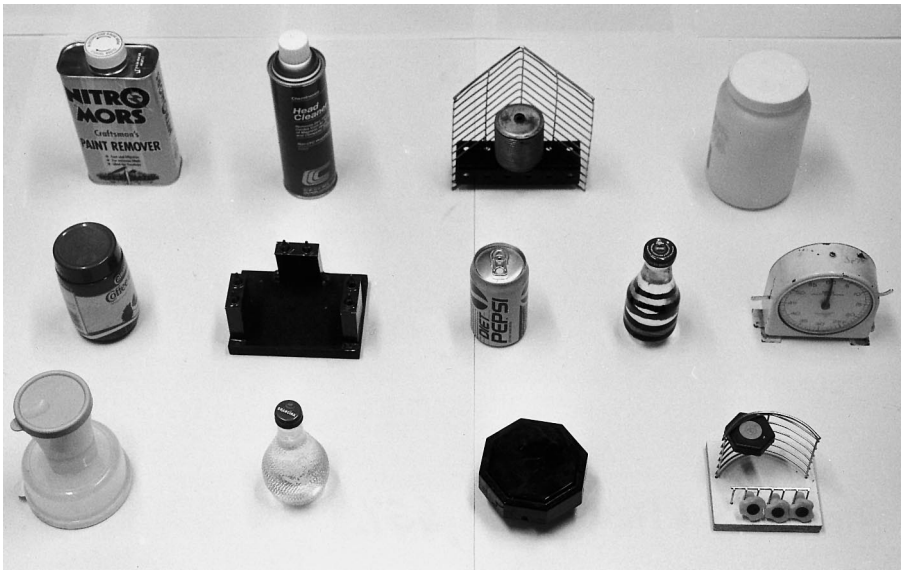

Fig. 1 Typical objects used in the experiments

A2) were placed in the box close (approximately 10 cm) to two adjacent corners, which were randomly selected for each rat. The rat was then placed in the box for 3 min and then returned to its cage. During the retention interval, the experimenter removed both objects and replaced them by their identical copies, one of which was placed in a previously used location in the box whereas the other was placed near to the other adjacent corner. As a consequence, when the rat was exposed to the two identical copies of the sample stimuli only one was in its original location (Af, object in its familiar location; An, object in a new location). The time spent exploring each object was again recorded. The task assesses the ability to discriminate the novelty of the object location, but the object itself and the test arena are already familiar to the rat.

Rats in experiment 1 received four sessions of the object recognition task: two sessions with a 1-min delay interval and then two sessions with a 15-min delay interval. Rats in experiment 2 received two sessions in the object recognition task and two sessions in the object location task. For both tasks in experiment 2 the delay interval was 15 min. The minimum interval between testing sessions was 48 h and all groups were tested concurrently. The delay of 15 min was selected to ensure that the task was sufficiently demanding, but not so long that floor effects might impede group comparisons (Ennaceur and Delacour 1988).

Measurements and statistical analysis

Paired Student's *t*-tests (two-tailed) were used to compare, within each of the separate groups, the time spent exploring each of the objects in the choice phase of the various conditions. It should be noted that object recognition and object location tasks are based on spontaneous exploratory activity, and as a consequence they do not exclude the possibility of individual animals having a preference for a specific object or place that is independent of the familiarity/novelty of that item. For this reason variance levels can alter markedly from condition to condition, and are often greatest in impaired groups.

In addition, analyses of variance were used to examine the following measures: (1) e1, the total time spent in exploring the two identical objects in the sample phase; (2) e2, the total time spent exploring the two objects in the choice phase; (3) d1, the index of discrimination, which is the difference in time spent exploring the two objects in the choice phase (e.g. B–A3 in the object recognition test and An–Af in the object location test); and (4) d2, the discrimination ratio, which is the difference in exploration time (i.e. d1) expressed as a proportion of the total time spent exploring the two objects in the choice phase (e.g. B–A3/B+A3 in the object recognition task and An–Af/An+Af in the object location task). When the results from two sessions using the same retention delay were comparable ($P>0.10$) their data were pooled and the analyses were run on these pooled data. This was consistently the case in the present study and so all repeated conditions have been pooled.

Table 1 Index of the different measures involved in the spontaneous recognition memory task for objects and location of objects [*e1* the total time spent exploring two objects A1 and A2 in the sample phase, *e2* the total time spent exploring objects B and A3 (object recognition) or objects An and Af (object location) in the choice phase, where A3 and Af represent copies of the sample objects (A1 and A2), B is a new object and An is a familiar object in a new location, *d1* the index of discrimination, i.e. the difference in time spent exploring the two objects in the choice phase (e.g. B–A3 in the object recognition task and An–Af in the object location task), *d2* the discrimination ratio, i.e. the difference in exploration time (i.e. d1) divided by the total time spent exploring the two objects in the choice phase (e.g. B–A3/B+A3 in the object recognition task and An–Af/An+Af in the object location task)]

Task	Variables			
	e1	e2	d1	d2
Object recognition	A1 + A2	A3 + B	B–A3	B–A3/B+A3
Object location	A1 + A2	Af + An	An–Af	An–Af/An+Af

Experiment 1

Materials and methods

Subjects

The study involved 41 naive male rats of the pigmented DA strain (Bantin and Kingman, Hull). Throughout the period of the experiment the animals were housed individually under diurnal conditions (14 h light/10 h dark), all testing occurring at a regular time during the light period. During the experiment the animals were supplied with food and water ad libitum. At the time of surgery the animals were aged 4–5 months and testing on the recognition task began a further 4 months after surgery.

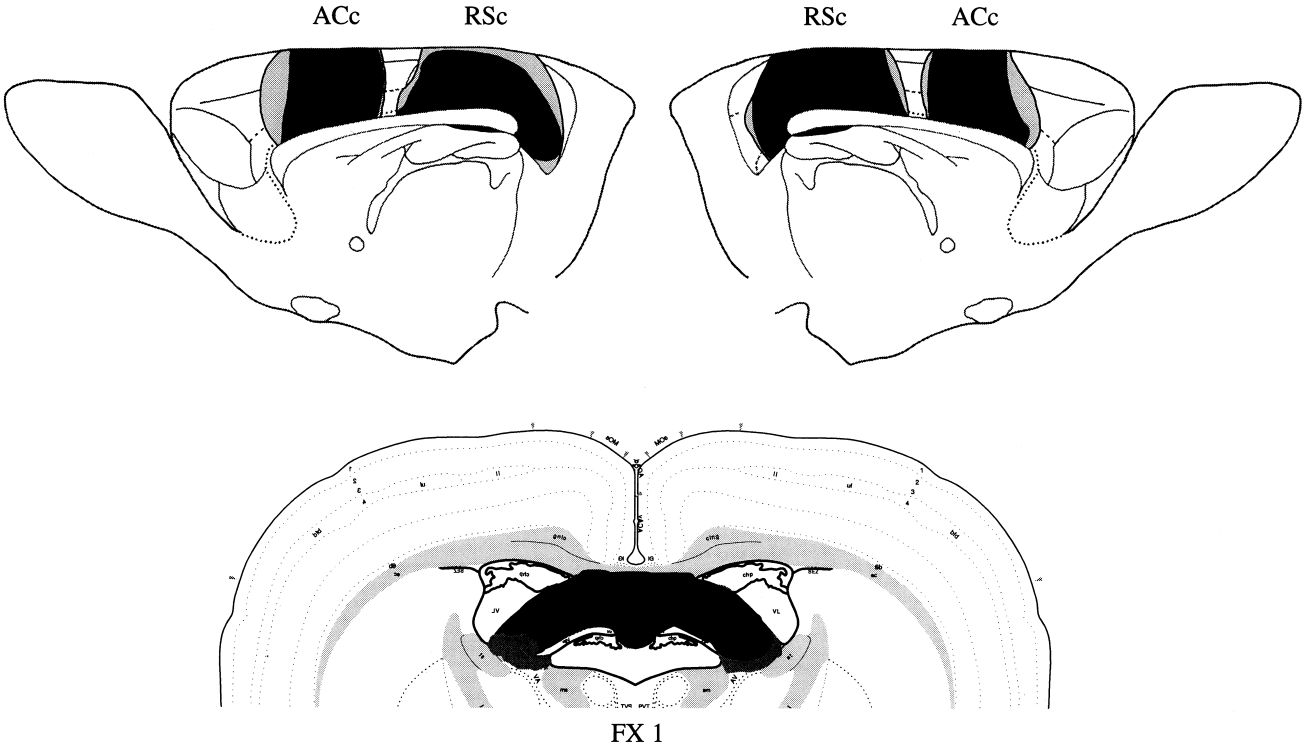

Fig. 2 The extent of the largest (*grey*) and smallest (*black*) lesions in the anterior cingulate (*ACc*), retrosplenial (*RSc*) and fornix (*Fx1*) groups in experiment 1. The cingulate lesions are shown on standard views of the medial surfaces of the brain, the fornix lesions are depicted on a coronal section

Surgical and histological procedures

The 41 rats were divided into four surgical groups: anterior cingulate cortex lesions (ACc, *n*=9), retrosplenial cortex lesions (RSc, *n*=10), fornix lesions (Fx1, *n*=8) and surgical controls for the ACc and RSc groups (CONT1, *n*=14).

Animals were anaesthetised by intraperitoneal injection (60 mg/kg) of pentobarbitone sodium. Each animal was then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga) and the scalp retracted to expose the skull. A craniotomy was then made above the sagittal sinus and the dura cut to expose the cortex above the target region.

For the ACc lesions, injections of 0.28 µl of 0.09 M NMDA (Sigma, Poole, UK) dissolved in phosphate buffer (pH 7.2) were made through a 1-µl Hamilton syringe into two sites in each hemisphere. The stereotaxic coordinates relative to ear-bar zero, with the incisor-bar set at +5.0 relative to the horizontal plane, were: AP +7.6, LAT 0.7 and AP +6.3, LAT 0.7. The depth at both sites was 2.2 mm below the top of the cortex. Each injection was made gradually over a 4-min period and the needle was left in situ for a further 4 min before being withdrawn.

The procedure for the RSc lesions was the same, only now the animals received three injections of 0.25 µl of 0.09 M NMDA per hemisphere. The coordinates of these injections, relative to ear-bar zero were: AP +3.7, LAT 0.7; AP +2.8, LAT 0.7; and AP +0.9, LAT 0.7. The two rostral injections were placed 1.9 mm below the cortex while the most caudal injection was placed 2.2 mm below the cortex. Those animals (CONT1) that were surgical controls for the ACc and RSc groups received a craniotomy and the dura was cut in the appropriate place. For half the CONT1 animals the placement matched that of the ACc lesions, while for the other half it matched the RSc lesions.

The initial stages of the Fx1 lesions were the same as those described above for the ACc and RSc lesions, but the actual lesion was made by radiofrequency. A Radionics TCZ (Radionics, Burlington) electrode (0.3 mm tip length, 0.25 mm diameter) was lowered vertically into the fornix and the tip temperature raised to 75°C for 60 s using an RFG4-A Lesion Maker (Radionics, Burlington). Two lesions were made in each hemisphere. The stereotaxic coordinates of the lesions relative to ear-bar zero were: AP +5.3, HT +7.1, LAT 0.7; and AP +5.3, HT 7.0, LAT 1.7. At the completion of all surgeries the animals received sulphanilamide powder and the skin was then sutured.

On completion of the experiment the animals were killed and perfused intracardially with physiological saline followed by 5% formol-saline. The brains were rapidly removed and placed in 5% formol-saline. Subsequently, the brains were blocked, embedded in wax (Paraplast), and cut into 10-µm coronal sections. Every tenth section was mounted and stained with cresyl violet, a Nissl stain.

Results

Histological analysis

Following histological analysis, one animal in the CONT1 group was excluded due to vascular damage in the cortex. One animal in the ACc group was also excluded as the lesion was unusually small.

Figure 2 shows the extent of the largest and smallest of the ACc lesions. These surgeries resulted in a localised region of cell loss that was always restricted to area 24 (Vogt 1981). Within the extent of the lesion there was a complete loss of cells. In contrast, there was no evidence of direct damage to the corpus callosum, the cingulum bundle or the fornix. While the lesions involved much of area 24a (Vogt 1981), they sometimes spared the most dorsal parts of area 24b (Fig. 2). In addition, the pregenual portions of the cingulate cortex and the posterior transitional region were often spared. There was no evidence of any thalamic degeneration.

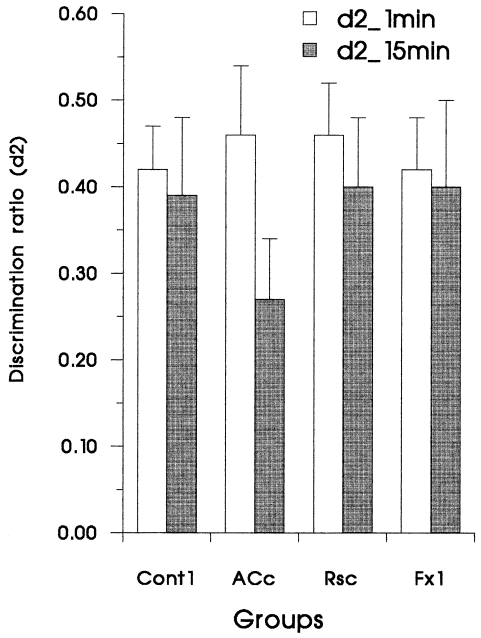

Fig. 3 Mean value (±SEM) of the discrimination ratio d2 in the object recognition task with 1-min and 15-min retention delays. There are no significant differences between groups (P>0.10)

The RSc lesions produced considerable damage to area 29 (Vogt 1981). All the surgeries resulted in extensive cell loss throughout the rostral and middle portions of the retrosplenial cortex, usually involving all of areas 29b, 29c and 29d. The lesions extended caudally behind the splenium both ventrally and laterally to reach the border of the subicular complex (areas 29a and 29b). While all the subfields of area 29 (a–d) were involved in the RSc lesions, the only parts of the retrosplenial cortex to be spared were the rostral transitional zone with area 24 and the most caudal regions behind the splenium. Diffusion of the injectate consistently resulted in a localised patch of cellular damage in that part of the CA1 field closest to the retrosplenial cortex. The cingulum bundle always appeared intact (Fig. 2). Finally, all RSc cases had a distinct region of cellular shrinkage and loss restricted to the anterior ventral nucleus of the thalamus.

The Fx1 lesions produced very extensive damage to the fibre tract itself and in most cases also extended into the most rostral head of the hippocampus. In six of the eight animals the tract was completely severed bilaterally while in the remainder only the most lateral tips of the fimbria were spared (Fig. 2). In nearly all cases the lesion, in addition to damaging a small part of the corpus callosum, involved the dorsal most limit of the anterior ventral and anterior dorsal thalamic nuclei.

Object recognition

Overall levels of exploration. Comparisons of the total time spent exploring the test objects in the 1-min and 15-min delay conditions indicated that there were no group differences for either the sample phase or the choice phase (P>0.10).

Discrimination performance. All groups spent more time exploring the new objects than the familiar objects in both the 1-min and 15-min delay conditions (highest level of probability for all within-group comparisons, P=0.01). Comparisons between groups using measures d1 (difference in total time exploring) and d2 (difference in time as a proportion of total time spent exploring) indicated that there was no group difference for measure in either the 1-min or 15-min delay conditions (all, P >0.10; Fig. 3).

Experiment 2

Materials and methods

Subjects

The study involved 47 male rats of the pigmented DA strain (Bantin and Kingman, Hull). The animals received either neurotoxic or radiofrequency lesions placed in either the medial prefrontal cortex (Pfc, n=13), the cingulate and retrosplenial cortices (Cg, n=8), the cingulum bundle (CB, n=8) or the fornix (Fx2, n=6). A surgical sham group was also included (CONT2, n=12). At the time of surgery the rats were aged 4 months (205–245 g), and the current experiment began 3 months later. Throughout the course of the experiment the animals had free access to food and water.

Surgical and histological procedures

The basic surgical and lesioning procedures matched those used in experiment 1. All the medial prefrontal lesions (Pfc) were centred in the prelimbic area but two different lesion coordinates were used. For the larger of the medial frontal cortex lesions (n=6) 0.28 µl of 0.09 M NMDA was injected into two different sites in each of two different vertical tracts, i.e. four injection sites per hemisphere. The stereotaxic coordinates relative to ear-bar zero, with the incisor-bar set at +5.0 relative to the horizontal plane, were: AP +8.7, LAT ±0.7, with depths of 4.5 mm and 2.2 mm below the top of the cortex; and AP +10.0, LAT ±0.7, with depths of 3.5 mm and 2.0 mm below the top of the cortex. The same procedure was used for the more restricted prefrontal lesions (n=7) except that only a single injection was made in each of the two tracts, and a total of 0.3 µl of 0.09 M NMDA was injected in each of these sites. The coordinates relative to ear-bar zero were: AP +8.7, LAT ±0.7, depth from the top of the cortex 3.5 mm; and AP +10.0, LAT ±0.7, depth from the top of the cortex 2.8 mm.

For the total Cg lesions 0.3 µl of 0.09 m NMDA was injected into five sites per hemisphere. The coordinates of these injections relative to ear-bar zero were, going rostral to caudal: AP +7.6, LAT ±0.7; and AP +6.0, +4.3, +2.6, +0.9, with the LAT being ±0.8 in all cases. The depth at each site, going rostral to caudal, was 2.0 mm, 2.0 mm, 1.7 mm, 1.1 mm and 2.2 mm from the top of the cortex.

The procedure for the CB lesions was very similar to that for the Fx1 animals except that the TCZ electrode was lowered vertically into three sites per hemisphere before the tip temperature was raised to 75°C for 60 s. The coordinates of these lesions, relative to ear-bar zero, were as follows: AP +7.9, LAT ±1.1; AP +3.7, LAT ±1.0; and AP +1.4, LAT ±0.9. The depths at the three sites, going rostral to caudal, were 2.1 mm, 1.7 mm and 1.9 mm below the top of the cortex. In five of the eight CB cases a further lesion (60 s, 75°C) was placed 0.4 mm dorsal to each of those already described (i.e. these animals received two lesions in each of the

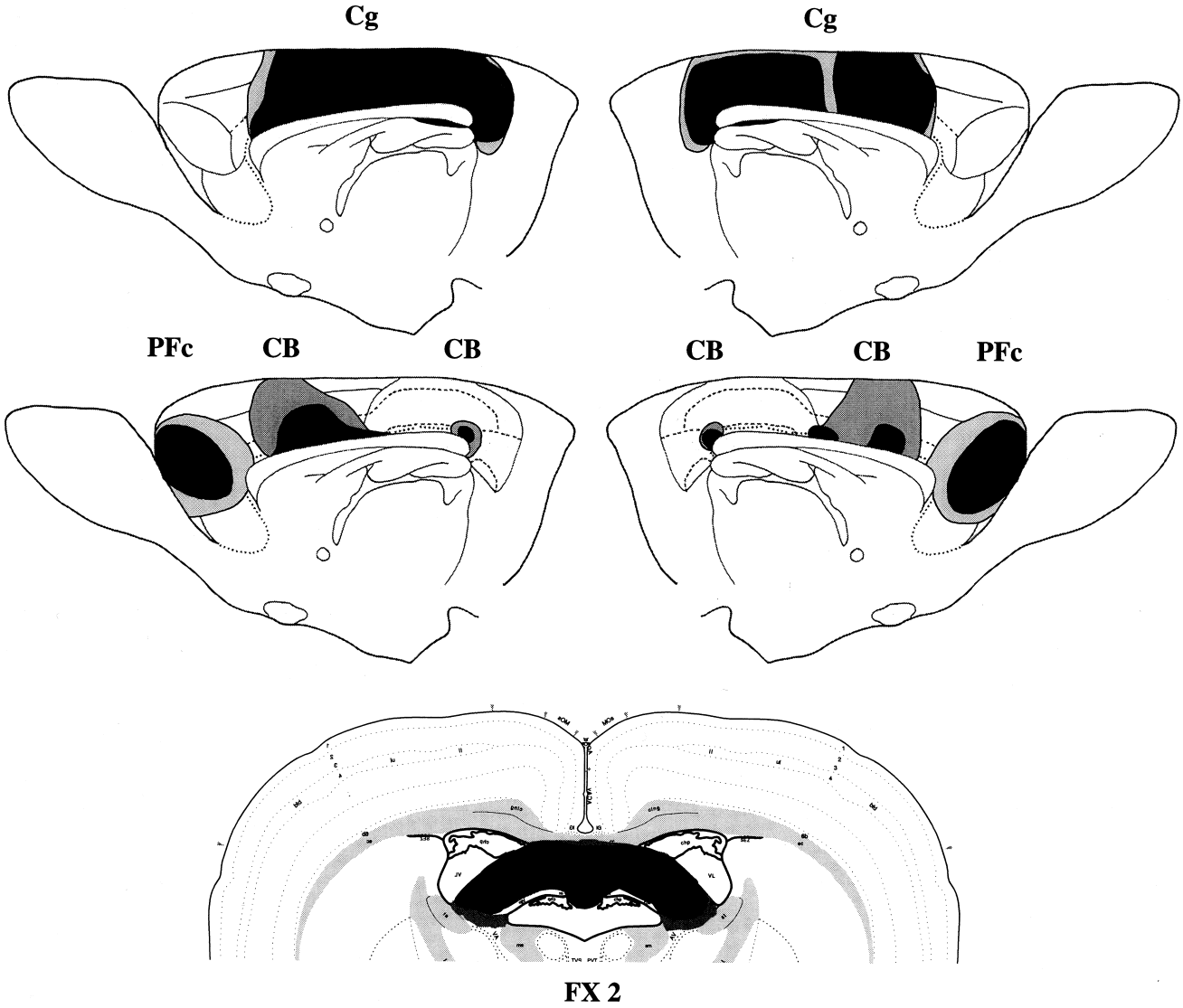

Fig. 4 The extent of the largest (*grey*) and smallest (*black*) lesions in the extensive cingulate cortex (*Cg*), medial prefrontal (*Pfc*), cingulum bundle (*CB*) and fornix (*Fx2*) groups in experiment 2. The cingulate lesions are shown on standard views of the medial surfaces of the brain; the fornix lesions are depicted on a coronal section. The cingulum bundle and prefrontal cortex lesions are depicted on the same medial views, the Pfc lesions being in the most rostral portion of the brain. (Although all CB animals received lesions at three sites per hemisphere, the more rostral two lesions often merged into one another)

three tracts per hemisphere). The surgeries for the Fx2 group were identical to those for Fx1. The animals acting as sham controls (CONT2) received the same initial surgeries as the Pfc animals, but although the dura was cut and retracted no injection of NMDA was made. On completion of the experiment the brains were processed as for experiment 1.

Results

Histology

Following histological examination none of the animals was discarded from their respective surgical groups. All the lesions in the Pfc group (Fig. 4) produced extensive, bilateral cell loss throughout the prelimbic region (area 32). In all but two cases the lesions extended rostrally to the frontal pole, while in all cases the lesions extended caudally to a level just in front of the genu of the corpus callosum. The induseum griseum was involved in the six cases with larger lesions. The infralimbic cortex was damaged bilaterally in all cases, but in two of the 13 Pfc cases this involvement was very minor. The lesions also extended dorsally above the prelimbic area to include the more rostral and ventral parts of the anterior cingulate area (ACAd), but in the majority of cases the cellular loss in ACAd was very slight.

The Cg lesions involved almost the entire extent of the cingulate (anterior cingulate and retrosplenial) cortices that lie dorsal to the corpus callosum (Fig. 4). The cell loss in the anterior cingulate cortex began at the genu and continued caudally to the retrosplenial cortex beyond the splenium. Throughout this level both the ventral and the dorsal parts of the retrosplenial cortex showed an almost complete loss of cells (Fig. 4), while more rostrally the lesions extended dorsally to include

ACAv, ACAd, and parts of the adjacent secondary motor area (Fig. 4). Caudal to the splenium there was often sparing of the dorsal retrosplenial cortex, but the involvement of the ventral retrosplenial cortex often continued to near its caudal limit (Fig. 4). Some cellular loss was observed in those parts of the hippocampal field CA1 in closest proximity to the ventral retrosplenial cortex (Fig. 4). In all cases there was evidence of cellular degeneration in the anterior ventral thalamic nucleus, although the cingulum bundle appeared to be intact.

The CB lesions involved three bilateral radiofrequency lesions placed at different AP levels. In all CB cases there was very considerable, bilateral damage to the bundle, at more than one level (Fig. 4). Very distinct cellular loss was always observed in the anterior ventral thalamic nucleus. The lesions did not involve the fornix, but in four cases there was very minor damage to the dorsal limit of the hippocampus (principally to the alveus) below the mid-AP lesion. In three of these four cases the hippocampal damage was only unilateral. The extent of damage to those parts of the anterior cingulate and retrosplenial cortices adjacent to the cingulum bundle sites was variable, but in the majority of cases the lesions were quite selective, with limited direct cortical damage (Fig. 4). This cortical damage was always considerably less than that in the Cg cases, and there were always regions of intact cortex between the three radiofrequency lesion sites.

In three of the six Fx2 lesions the tract was completely transected. In two of the remaining animals the fimbria/fornix was completely cut in one hemisphere and only the most lateral tips of the fimbria were spared, while in one case the most lateral tips of the fimbria were spared bilaterally (Fig. 4). In four of the Fx2 cases there was very slight involvement of the most dorsal limits of the anterior ventral and anterior dorsal thalamic nuclei.

Object recognition

Initial analyses found no differences between the two groups of Pfc animals (i.e. those with larger lesions and smaller lesions) for either the object recognition task or the object location task. These animals were therefore combined to form a single group throughout.

Overall levels of exploration. Comparisons of the total time spent exploring the test objects indicated no significant group difference in either the sample phase or the choice phase (P>0.10).

Discrimination performance. Within-group analyses showed that all five groups spent more time exploring the new object than the familiar one (highest P=0.02). Furthermore, between-group comparisons found no evidence of a lesion effect on the level of discrimination using measures d1 or d2 (both P>0.10; Fig. 5).

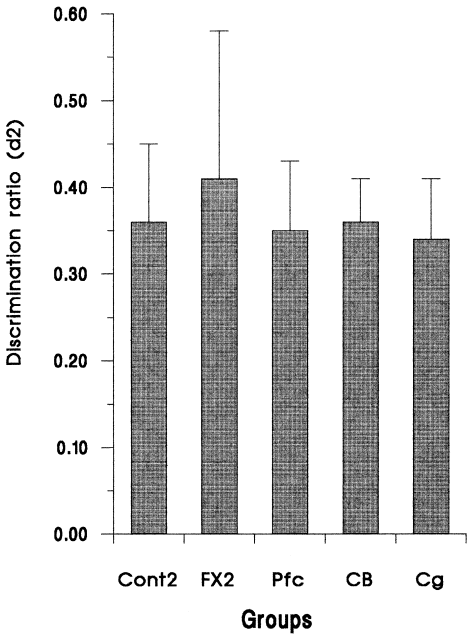

Fig. 5 Mean value (±SEM) of the discrimination ratio d2 in the object recognition task with 15-min retention delay. There were no significant differences between groups (P>0.10)

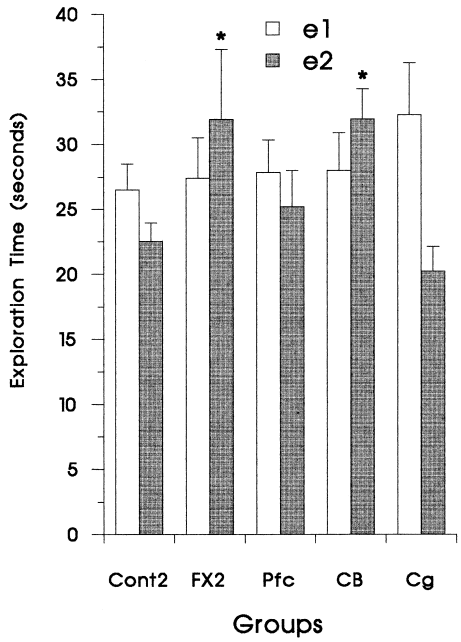

Fig. 6 Mean value (±SEM) of the total time rats spent exploring both objects in the sample phase (e1) and the choice phase (e2) in the object location task. * P<0.05 compared with CONT2 and Cg groups

Object location

Overall levels of exploration. There were no group differences in the amounts of total exploration in the sample phase [e1: F(4,42)=0.57, P>0.10]. A significant group effect was, however, found for the total amount of exploration during the choice phase [e2: F(4,42)=3.32,

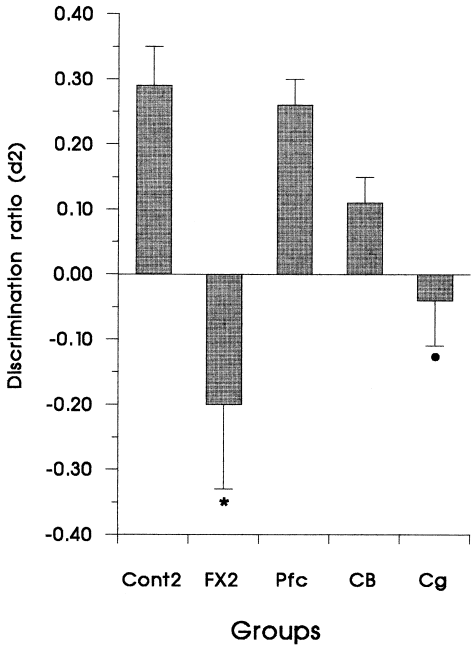

Fig. 7 Mean value (±SEM) of the discrimination ratio d2 in the object location task with 15 min retention delay. * $P<0.05$ compared with CONT2, Pfc, CB and Cg groups; • $P<0.05$ compared with CONT2 and Pfc and Fx2 groups

$P \leq 0.02$]. Subsequent Newman-Keuls tests showed that the Fx2 and CB groups spent more time exploring the two objects than the CONT2 and Cg groups ($P \leq 0.05$).

Discrimination performance. Within-group analyses showed that the CONT2, Pfc and CB rats all spent significantly more time in exploring the object in the new location compared with the time they spent with the object in its original location (d1, highest $P=0.02$). In contrast, the Fx2 and Cg groups failed to discriminate between the two locations. In fact, the Fx2 group spent more time overall exploring the object in its original location (Fig. 7). This reverse preference was, however, largely due to one Fx2 rat that spent 44 s exploring the object in the familiar location and no time in the new location. Taken as a group, the Fx2 animals failed to discriminate the change in location, as they showed no significant preference for either the new or the old location. An analysis of variance using the discrimination scores showed a significant group effect for d1 [$F(4,42)=7.37$, $P=0.0001$] and d2 [$F(4,42)=8.97$, $P<0.0001$]. Newman-Keuls tests helped to confirm that these group effects reflected the unusual level of discrimination demonstrated by the Fx2 group and the low level of discrimination demonstrated by the Cg group ($P<0.05$). Thus for both discrimination scores (d1 and d2) the Fx2 group was significantly different from the CONT2, Pfc and CB groups ($P<0.05$). It showed a high level of discrimination in the opposite direction to the other groups, but the Fx2 groups d1 scores are not significantly different from zero ($P>0.10$). In addition, the Cg group had lower scores than the Pfc group for d1, and lower scores than the CONT2 and Pfc groups for d2. The Fx2 scores for d2 were significantly different from those of the Cg group.

Discussion

One of the main findings of the current study was that an array of lesions in limbic sites connected with the hippocampus did not disrupt performance on a test of object recognition, even though the task used retention delays of up to 15 min. Thus, it was found that rats with cytotoxic lesions of the anterior cingulate cortex, the retrosplenial cortex, the anterior cingulate and retrosplenial cortices combined, or the medial prefrontal cortex all showed normal levels of preference for novel objects. Similarly, lesions transecting the fornix or the cingulum bundle spared performance on the same task. A different pattern of results was found, however, on a test of object location memory. This task, which took advantage of the preference normal rats show for an object that has moved from its previous position, revealed an impairment following radiofrequency lesions of the fornix and cytotoxic lesions of the cingulate cortex. In contrast, rats with lesions in the medial prefrontal cortex or the cingulum bundle were still able to identify the object that was in a novel position.

Before discussing the implications of the normal recognition performance by the various lesion groups, it is important to consider first the validity of the findings. It seems most unlikely that the lack of any lesion effect was a consequence of incomplete surgeries. In the case of the tract lesions (Fx1, Fx2 and CB), both the fornix and the cingulum bundle were often completely transected. Furthermore, the CB lesions were placed at several levels. It is also the case that the normal performance of both Fx groups (Fx1 and Fx2) matched a previous study which also found that fornix lesions did not disrupt preference for novel, complex objects (Ennaceur and Aggleton 1994). Similarly, the cytotoxic lesions were all placed in the target sites, and the injections of NMDA resulted in a total loss of neurons in many areas. The Cg lesions in experiment 2 were especially extensive, and although the Pfc lesions were relatively discrete, they consistently destroyed the principal target region, the prelimbic area.

It is also unlikely that the lack of any effect on the object recognition tasks merely reflects test insensitivity. Previous studies using this paradigm have shown that performance is consistently affected by delays (Ennaceur and Delacour 1988; Ennaceur and Aggleton 1994) and by systematic modifications in the similarity of the test items (Ennaceur and Aggleton 1994). It is also the case that a number of drug and lesion procedures can disrupt object recognition when they are tested using this same task (Ennaceur and Delacour 1987; Ennaceur et al. 1989, 1966a,b; Ennaceur 1991; Wood and Phillips 1991; Ennaceur and Meliani 1992a,b; Mickley et al. 1994; Scali et al. 1994; Cobb et al. 1995; Giovannelli et al. 1995; Hlinak and Krejci 1995; Bartolini et al. 1996). Indeed, it

has been argued that spontaneous tests of visual recognition may be especially sensitive to hippocampal damage (Alvarez et al. 1995). Although the present tests were not conducted immediately after surgery, the intention was to look for permanent deficits (Alvarez et al. 1995) and so this is not seen as a shortcoming.

The present finding, that lesions in a number of regions closely connected with the hippocampus need not disrupt familiarity judgements, can be combined with an array of other, related evidence. Studies using the same preferential exploration task have found that lesions neither of the medial septum nor the anterior thalamic nuclei affect recognition (Ennaceur and Meliani 1992a; Aggleton et al. 1995a). It has also been found that lesions of the fornix, the mammillary bodies and the medial prefrontal cortex do not affect the performance of delayed nonmatching-to-sample (DNMS) tasks by rats (Aggleton et al. 1990; Rothblat and Kromer 1991; Shaw and Aggleton 1993). All these recognition tasks use complex objects as test stimuli, and so it appears that none of these regions is required for judging object familiarity even though all are closely linked with the hippocampus. This result is most striking in the case of the fornix lesions, as fornix damage so often mimics the effects of hippocampal damage. This pattern of results suggests one of two possibilities: first, that these particular hippocampal connections are not required and that familiarity judgements depend on hippocampal/temporal cortical interactions (Squire and Zola-Morgan 1991); second, that the rat hippocampus itself is not required for judging the familiarity of complex objects. Support for this second view comes from studies using a variety of DNMS tasks which have found that even very extensive hippocampal lesions can spare object recognition performance (Aggleton et al. 1986; Mumby et al. 1995) or produce only very mild deficits (Mumby et al. 1992; Steele and Rawlins 1993). Similarly, electrophysiological recordings have typically failed to find neurons in the hippocampus that respond in a consistent manner to novel objects (Zhu et al. 1995a). It is also the case that while induction of c-*fos*, a marker for active neurons, is observed in a number of cortical sites in rats following exposure to novel objects, these changes do not appear to be present in the hippocampus (Zhu et al. 1995b).

Taken together, these findings indicate that the integrity of the hippocampus and an array of interconnected limbic structures is often, not necessary for judging familiarity. It is, however, important to note that all the studies cited so far have used discrete complex objects and there is evidence that lesions of the hippocampus or the fornix can disrupt the recognition of other types of stimuli (Olton and Feustle 1981; Raffaele and Olton 1988; Rawlins et al. 1993). While these exceptions need to be more fully characterised, it appears that the hippocampus/fornix is required when the tasks involve relatively large, featureless stimuli (Rawlins et al. 1993; Cassaday and Rawlins 1995). One possible explanation is that the hippocampus, and probably some related limbic regions, are involved in those recognition tasks in which the stimulus is treated in the same way as a spatial array rather than as a discrete object.

Consistent with this view was the finding from experiment 2 that the animals with fornix lesions seemed unable to distinguish the object that was in a novel location. This deficit accords with many other studies of spatial working memory (Olton et al. 1979), and in fact the same Fx2 animals were impaired on two other tests of spatial working memory: delayed alternation in a T-maze and delayed nonmatching-to-position (Neave et al. 1994; Aggleton et al. 1995). These findings reinforce the dissociation between spatial working memory and object recognition that has been observed in other studies (Aggleton et al. 1986; Hunt and Aggleton 1991; Ennaceur and Meliani 1992a,b; Shaw and Aggleton 1993).

The extensive Cg lesions also disrupted the object location task. Although this finding appears consistent with the effects of conventional lesions of the cingulate cortices on spatial memory tasks (Sutherland et al. 1988; Markowska et al. 1989; Sutherland and Hoesing 1993), a number of recent studies using neurotoxic agents have questioned the magnitude of these lesion effects and suggested an important contribution from cingulum bundle damage (Meunier and Destrade 1988; Neave et al. 1994; Aggleton et al. 1995b). Indeed, the Cg and the CB groups in the current study were tested on two other tests of spatial working memory. Neither lesion affected delayed nonmatching-to-position (DNMP) in an operant chamber, but the CB and not the Cg group was impaired on T-maze alternation (Aggleton et al. 1995b). The opposite pattern was found in the current study: CB animals spent significantly more time exploring the new rather than the familiar location (Student's t-*t*est on d1) and their level of discrimination (d2) was not different from that of the control cases, whereas Cg animals did not discriminate between the locations and their level of discrimination (d2) was significantly different from that of the controls. While these results need to be confirmed with larger group sizes, we suggest that the different pattern of results obtained in the present and previous study (Aggleton et al. 1995b) underlines the existence of potentially important distinctions within the domain of spatial working memory. In the present case, the dissociation may reflect a distinction between those tasks which can be solved by the animal only having to remember where it has most recently been or where it has to reach, and those tasks in which the location of a separate, distinct item is to be remembered (Ennaceur 1995a,b; Ennaceur et al. 1996c). This proposal requires further examination, but it may help to explain why retrosplenial cortex lesions can disrupt certain visual-spatial conditional tasks as successful performance will involve linking the location of the correct response to the specific, appropriate stimulus (Bussey et al. 1996). It remains unclear why the CB group animals were unimpaired on the object location task, as the surgery should have disconnected much of the cingulate cortex. This may reflect the sparing of very particular connections, but it should also be noted that neither of the Cg discrimination scores (d1

or d2) for the object location task differed significantly from those of the CB group. In contrast, at least one of these Cg discrimination measures differed significantly ($P<0.05$) from the remaining three groups (Pfc, Fx2, CONT2).

Finally, the medial prefrontal lesions were found to have no effect on the object location task, in spite of the fact that the same lesions markedly impaired DNMP performance (Aggleton et al. 1995b). In addition, medial prefrontal cortex lesions in other studies have been found to disrupt various spatial working memory tasks (Brito et al. 1982; Kesner et al. 1989; Brito and Brito 1990; Dunnett 1990; Shaw and Aggleton 1993). It is, however, most likely that this apparent discrepancy arises from the fact that the design of the present preferential task helps to minimise a number of other factors that can disturb performance, i.e. it provides a purer test of spatial working memory. Consistent with this is the fact that careful analysis of the DNMP scores shown by the Pfc animals indicates that their deficit principally reflected problems of perseveration (Aggleton et al. 1995b). It is also the case that other allocentric spatial working memory deficits associated with prelimbic damage can be transient in nature (Thomas and Brito 1980; Shaw and Aggleton 1993), again suggesting that the primary deficit is not one of allocentric spatial processing per se. Such issues serve to highlight the value of the spontaneous preference tasks used in the current study, as such tasks can examine different aspects of working memory and yet be relatively free from other, potentially confounding demands.

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