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Removal of cholinergic input to perirhinal cortex disrupts object recognition but not spatial working memory in the rat

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Abstract

The perirhinal cortex of the temporal lobe has a crucial role in object recognition memory. Cholinergic transmission within perirhinal cortex also seems to be important for this function, as the muscarinic receptor antagonist scopolamine disrupts object recognition performance when administered systemically or directly into perirhinal cortex. In the present study, we directly assessed the contribution of cholinergic basal forebrain input to perirhinal cortex in object recognition. Selective bilateral removal of the cholinergic basal forebrain inputs to perirhinal cortex was accomplished by injecting the immunotoxin 192 IgG-saporin directly into perirhinal cortex in rats. These animals were significantly impaired relative to vehicle-injected controls in a spontaneous object recognition task despite intact spatial alternation performance. These results are consistent with recent reports of object recognition impairment following acute cholinergic receptor blockade and extend these findings by demonstrating that chronic removal of cholinergic basal forebrain input to an otherwise intact perirhinal cortex causes a severe object recognition deficit similar to that associated with more extensive cell body lesions of perirhinal cortex.

Introduction

The perirhinal cortex (PRh) is critical for visual recognition memory in humans (Buffalo *et al*., 1998), monkeys (Meunier *et al*., 1993; Suzuki *et al*., 1993; Gaffan, 1994) and rats (Mumby & Pinel, 1994; Ennaceur *et al*., 1996; Bussey *et al*., 1999; Winters *et al*., 2004a). Moreover, the neurotransmitter acetylcholine (ACh) may play an important role in recognition memory processes. Systemic administration of the cholinergic muscarinic receptor antagonists scopolamine and atropine can disrupt visual recognition in humans (Robbins *et al*., 1997), monkeys (Penetar & McDonough, 1983; Aigner & Mishkin, 1986; Aigner *et al*., 1991b) and rats (Huston & Aggleton, 1987; Bartolini *et al*., 1996; Vannucchi *et al*., 1997; Pitsikas *et al*., 2001). Furthermore, systemic treatment with the acetylcholinesterase (AChE) inhibitor physostigmine can facilitate performance on visual recognition tasks in monkeys (Aigner & Mishkin, 1986) and humans (Furey *et al*., 2000a), and administration of either of the AChE inhibitors metrifonate or tetrahydroaminoacridine attenuates the spontaneous object recognition deficit seen in aged rats (Scali *et al*., 1997a,b).

Recently, the effects of cholinergic agents in visual recognition tasks have been more directly linked to their possible actions in PRh. Specifically, microinfusions of scopolamine into PRh have been shown to impair visual delayed nonmatching to sample in monkeys (Tang *et al*., 1997) and spontaneous object recognition in rats (Warburton *et al*., 2003; Abe *et al*., 2004). Despite these results, however, the role of cholinergic basal forebrain projections in object recognition remains to be clarified, as excitotoxic lesions have been reported to impair performance in some cases, but not in others (Aigner *et al*., 1987, 1991a; Voytko *et al*., 1994; Bartolini *et al*., 1996). Moreover, a direct role in object recognition for cholinergic basal forebrain input to PRh has yet to be demonstrated.

Thus, in the present study, we made selective lesions of the cholinergic basal forebrain input to PRh by infusing the cholinergic immunotoxin 192 IgG-saporin directly into PRh in rats (Wiley *et al*., 1991; Holley *et al*., 1994; Ohtake *et al*., 1997; Bucci *et al*., 1998; Dougherty *et al*., 1998; Winters *et al*., 2004b). This technique allows us to compare the effects of cholinergic pathway ablation with findings from intra-PRh scopolamine infusions (Tang *et al*., 1997; Warburton *et al*., 2003; Abe *et al*., 2004). This is an important issue because the behavioural effects of cholinergic denervation using 192 IgG-saporin in other areas, such as the hippocampus, are not always consistent with those following transient cholinergic receptor blockade in the same regions (e.g. Dunnett *et al*., 1990; Winters & Dunnett, 2004). Such findings suggest that, although septohippocampal ACh is often involved in hippocampus-mediated spatial learning and memory, it is not necessary for these functions (Parent & Baxter, 2004). Impairment in object recognition memory following 192 IgG-saporin lesions is therefore required to show unequivocally that the basal forebrain cholinergic input to PRh is necessary for this type of memory. Here we show that such a lesion does, indeed, disrupt object recognition despite leaving spatial working memory intact.

Materials and methods

Subjects

Thirteen young adult male Lister Hooded rats (Charles River, UK), weighing 350–450 g prior to surgery were housed in groups of four per cage in a room with a 12–12-h light–dark cycle (lights on at 19:00 h). All behavioural testing was conducted during the dark phase of the cycle. During testing, rats were fed approximately 15 g of laboratory chow following daily behavioural sessions to maintain

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weights at 85–90% of free-feeding body weight. Water was available *ad libitum* throughout the experiment. All experimentation was conducted in accordance with the UK Animals (Scientific Procedures) Act 1986.

Surgery

For all surgeries, rats were anaesthetized with an intraperitoneal injection (60 mg/kg) of sodium pentobarbital (Sagatal, Rhône Mérieux, UK). Animals were allocated to one of two groups for surgery. One group (SAP-PRh, $n = 7$) received bilateral injections of 192 IgG-saporin (Chemicon International, Harrow, UK; 0.02 μg/μL in Dulbecco's saline) at the following three sites within PRh, with anterior (A) measured from bregma, lateral (L) measured from midline, vertical (V) from the surface of the skull at the site of injection, and the incisor bar set so that bregma and lambda were in the same horizontal plane: A = −3.8 mm, L = ± 4.8 mm, V = −7.3 mm; A = −5.3 mm, L = ± 4.8 mm, V = −7.3 mm; and A = −7.3 mm, L = ± 4.8 mm, V = −6.8 mm. All injections were delivered manually using a 1-μL Hamilton syringe with the syringe orientated at 20° from vertical. For each injection, a total of 0.2 μL of toxin was delivered over 3 min, and four additional minutes were allowed for diffusion before the cannula was withdrawn. A second group of rats (Control, $n = 6$) underwent an identical procedure but received equal volume injections of saline.

Histology

One week following the completion of behavioural testing, rats were deeply anaesthetized with 2 mL of sodium pentobarbitone (Euthatal, 200 mg/mL; Rhône Mérieux). They were then perfused transcardially with 100 mL of 0.01 M phosphate-buffered saline (PBS, pH 7.4), followed by 250 mL of 4% paraformaldehyde (pH 7.4). The brains were removed, postfixed in 4% paraformaldehyde at 4 °C for 24 h and then immersed in 25% sucrose in PBS until they sank. Frozen 40-μm sections were cut on a sledge microtome. Four parallel series of one in every five sections through the basal forebrain and PRh were analysed. One series was stained for Nissl substance with Cresyl Violet to visualize cell bodies and analyse possible nonspecific damage in the PRh. A second series was mounted and stained for AChE activity using a modified thiocholine silver precipitation method (Koelle, 1955), which was examined visually to determine the extent of cortical cholinergic denervation. The third series was processed for choline acetyltransferase (ChAT) immunoreactivity to visualize cholinergic cell bodies in the basal forebrain, and a fourth series was immunostained for parvalbumin (PV), which is colocalized with gamma-amino-butyric acid (GABA) in the rat basal forebrain (Freund, 1989). Briefly, for these latter two series, free-floating sections were stained in 15-mL stoppered pots on an orbital shaker. Following blocking in a solution of normal goat serum (30 μL/mL; Sigma, UK), the sections were incubated overnight at room temperature in the primary antibody at a dilution of 1 : 2000 for ChAT (Chemicon International) and 1 : 1000 for PV (Sigma) in 1% normal goat serum. The following day, sections were incubated in secondary antibody (1 : 200) and avidin–biotin complex (Vector, USA) solutions before being reacted in a 3–3′-diaminobenzidine (Sigma) demonstration of horseradish peroxidase until they turned a light brown. The sections were then washed in Tris nonsaline (×3) to end the reaction before being mounted and coverslipped. ChAT- and PV-immunoreactive (IR) cell bodies were counted throughout the following basal forebrain regions: the medial septum (MS), the vertical and horizontal limb nuclei of the diagonal band (VDB and HDB), and the nucleus basalis magnocellularis (NBM). Cells were counted in the MS, VDB, and HDB in the last six sections rostral to the crossing of the anterior commissure. The subpallidal NBM, which has been identified as an area of projection to the PRh (Woolf *et al*., 1984), was analysed for immunoreactive cells in four sections, starting with the first section caudal to the crossing of the anterior commissure. PV-IR cells were also counted within PRh as an index of possible disruption of intrinsic cortical cells resulting from intra-PRh injections of 192 IgG-saporin. These were counted in three sections per brain, at levels corresponding roughly to 3.8, 5.3 and 7.3 mm posterior to bregma (Paxinos & Watson, 1997). All cell counts were done manually using the 100× magnification on a Leitz Dialux microscope. These analyses were conducted blind to the experimental treatment.

Spontaneous object recognition

Spontaneous object recognition was conducted in a triangular arena, the walls of which were clear Perspex panels, each measuring 30 × 75 cm. The arena was placed on the floor of the testing room. The floor and walls of the arena were wiped down with a dry paper towel between rats, but otherwise were not cleaned during the experiment. The room contained features such as a door, light fixtures, unused, clean operant boxes, and a large white screen, which concealed the experimenter during testing. Data were collected by scoring exploratory bouts using a personal computer running a program written in Quickbasic 4.5. A video camera was mounted above the apparatus to record all testing sessions. Triplicate copies were obtained of the objects, which were made of glass, plastic or metal. For any given test, the pairs of objects were typically composed of the same material so that they could not readily be distinguished by tactile or olfactory cues. The height of the objects ranged from 5 to 24 cm, and all objects were affixed to the floor of the apparatus with Blu Tack (Bostik, Stafford, UK) to prevent them from being displaced during a testing session. As far as could be determined, the objects had no natural significance for the rats, and they had never been associated with a reinforcer.

All rats were habituated in two sessions in which they were allowed to explore the empty triangular arena for 20 min on two consecutive days. Testing began 24 h after the second habituation session. Rats experienced a single object recognition trial each day for four consecutive days (24 h apart). On each day, a different object pair was used for each rat, and the order of exposure to object pairs as well as the designated 'sample' and 'novel' objects for each pair were counterbalanced within and across groups. An experimenter, blind to the group membership of the animals, assessed the time spent exploring the objects during these sessions. The experimenter stood behind a large white screen to limit the distraction of the animal being tested.

The object recognition task is based on the tendency of rats to explore novel objects more than familiar objects. A test session consisted of two phases. In the sample phase, two identical objects (A1 and A2) were placed in the far corners of the arena. The rat was then placed in the remaining corner (the 'start corner'), facing away from the objects. The cumulative duration of exploratory bouts, the beginning and end of which were indicated by pressing a given key on the computer keyboard, was calculated by the computer program. Exploration of an object was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. Sitting on the object was not considered exploratory behaviour. The sample phase ended when the rat had explored the identical sample objects for a total of 25 s or when 5 min had passed, whichever

occurred first. At the end of the sample phase, the rat was removed from the arena and placed in a holding box in a separate room for a 15-min delay. After the delay, the rat was reintroduced to the arena (in the start corner, facing away from the objects) for the choice phase. The arena now contained a copy of the sample ('familiar') object (A3) in one far corner and a new object (B) in the other corner. Both objects were washed with a damp cloth during the delay to minimize the influence of olfactory cues. The corners into which the choice objects were placed were counterbalanced between rats and across sessions. The experimenter scored object exploration for 3 min, after which the rat was removed and returned to its home cage. The objects were washed thoroughly and the arena walls and floor wiped down between sessions. Separate records were taken of the exploration time for the novel and familiar objects. From these results we calculated a discrimination ratio, the proportion of total exploration time spent exploring the novel object (i.e. the difference in time spent exploring the novel and familiar objects divided by the total time spent exploring the objects), for the first minute of the choice phase, in which discrimination performance is typically maximal (Dix & Aggleton, 1999), on each object recognition trial. This measure takes into account individual differences in the total amount of exploration time.

Cross-maze spatial alternation

Spatial working memory was tested in a maze consisting of four wooden arms in the shape of a cross. Each arm was 80 cm long (measured from the intersection of the maze) and 12 cm wide and was separated from the immediately adjacent arm by 90°. The arms of the maze were 50 cm above the floor and each was bordered by a 2-cm rim to prevent rats from leaping from the maze. At the end of each arm was a food well 4.5 cm in diameter and 3 cm deep. Large wooden blocks ($10 \times 25 \times 6$ cm) were used to prevent access to certain arms of the maze at various times during testing. Normal overhead lighting was used for all phases of testing in the maze. Cross-maze testing was conducted in a typical experimental room containing a sink, bench area, cupboards and posters on the walls.

Testing began approximately 3 weeks following the end of the object recognition phase. Rats received several days of pretraining in which the cereal reward (Honey Nut Cheerios, Nestlé, Croydon, UK) was placed in the food wells of the maze arms, and rats were allowed to explore the maze to retrieve the reward. This phase was followed by eight daily sessions of eight trials. Each trial consisted of two parts – a sample run and a choice run. At the start of each trial, pieces of cereal were placed in the food wells of the designated choice arms, and a wooden barrier was used to block access to one of these arms (in addition to the fourth arm). On the sample run, the rat was placed at the end of the designated 'sample start arm', which was constant throughout the experiment. Because of the barrier, the animal could access only the one open arm, where it was confined for approximately 10 s while it ate the cereal at the end of the sample run. The rat was then taken from the sample arm while the barrier to the opposite arm was removed. The delay between the sample and choice runs was just long enough to remove the barrier from the blocked arm ('minimal' delay). The rat was placed briefly into a holding box during this period. The rat was then placed back into the maze with both choice arms now accessible for the choice run, on which it was required to choose the arm not visited on the sample run in order to obtain the reward. However, to encourage the use of allocentric spatial cues (rather than simply alternating based on egocentric, right–left responses), on half of the trials, the start arm for the choice run was the arm opposite the one used as the start arm for the sample run (i.e. the fourth arm). Thus, animals could alternate correctly if they used allocentric cues, because the location of the correct choice arm relative to these cues did not change between the sample and choice runs. On the other half of the trials, rats were started from the same arm for sample and choice runs. A wooden barrier always blocked the arm immediately opposite the start arm on choice runs. A choice was considered to have been made when the rat placed a hind foot into the arm. If the rat chose the arm not visited on the sample run then it was allowed to eat the reward before being returned to a holding cage until its next trial. If the wrong arm was chosen, i.e. the sample arm, the rat was confined to that arm for approximately 10 s before being returned to the holding cage. The rats were tested serially in groups of four, so that the intertrial interval was approximately 3–4 min. Each day contained a pseudo-random sequence of correct choices between the two arms (four trials each).

Following the initial eight sessions of minimal delay testing, rats were tested in a single eight-trial session with a delay of 15 s between the sample and choice runs. The next day they were assessed on a further eight trials with a delay of 5 min. In all phases, sessions consisted of four trials on which the choice run was started from the same arm as the sample run and four on which the choice run began from the opposite arm. The order of trial types was randomly determined for each session. The intertrial interval for the delay sessions was approximately 3–4 min, and rats were placed in a holding cage during the delay and intertrial intervals. For all phases of spatial alternation testing, the number of correct trials out of the total eight within each session was the primary dependent variable. These values were transformed to percentage correct scores for analysis.

Data analysis

Group means of three measures taken from object recognition testing (duration of the sample phase, object exploration time in the first minute of the choice phase and the discrimination ratio for the first minute of the choice phase) were submitted to independent-samples Student's *t*-tests. For cross-maze testing, the group means for the percentage of trials correct from each delay phase were submitted to a two-way (Group × Delay) analysis of variance (ANOVA) with repeated measures. Finally, average cell counts for each brain region considered (MS, VDB, HDB, NBM for ChAT; MS, VDB, HDB, NBM, PRh for PV) were subjected to Abercrombie's correction factor (Abercrombie, 1946) before analysis by separate two-way (Group × Region) ANOVAs, one for ChAT and one for PV. All analyses were conducted with a significance level of 0.05.

Results

Histology

Sections were analysed qualitatively for AChE staining in the cortex. Intracortical injections of 192 IgG-saporin resulted in markedly lighter AChE staining within PRh in all SAP-PRh rats. This difference is illustrated in Fig. 1A and B, which show typical AChE staining patterns in PRh of a Control and SAP-PRh brain, respectively. The darker and laminar staining pattern of AChE in the vehicle-injected PRh is absent in the cortex injected with 192 IgG-saporin. This attenuation of AChE fibre staining was seen in all SAP-PRh brains and was apparent throughout the rostral–caudal extent of PRh, extending from approximately −2.0 mm to approximately −7.8 mm from bregma in all animals (Paxinos & Watson, 1997). In addition to PRh alterations in AChE staining, three rats had lighter staining bilaterally in the subjacent entorhinal cortex, and another four rats

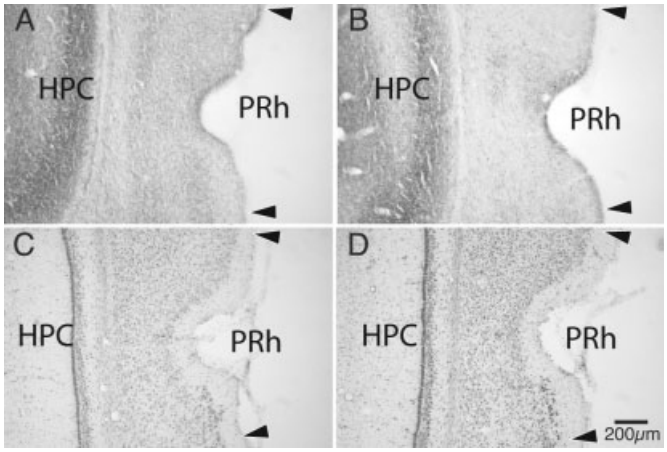

FIG. 1. Photomicrographs (4×) of representative sections taken from a Control (A and C) and SAP-PRh brain (B and D) at approximately 6.04 mm posterior to bregma. (A) AChE fibre staining from a Control brain illustrating typical moderately dense shading in PRh. (B) AChE staining in approximately the same region of PRh from a SAP-PRh brain. Note the attenuation of staining in this section caused by intracortical injections of 192 IgG-saporin. (C and D) Cresyl Violet staining was similar throughout PRh in Control and SAP-PRh sections. Arrowheads indicate the approximate boundaries of PRh (Burwell, 2001). HPC, hippocampus.

showed unilateral changes in this area. Two of the seven SAP-PRh brains also had lighter AChE staining bilaterally within Area TE3 just dorsal to PRh. Subsequent analyses revealed that bilateral changes in areas other than PRh were not differentially associated with behavioural deficits. AChE staining within the remainder of the neocortex and hippocampus was similar in Control and SAP-PRh brains.

The number of ChAT-IR cells within each of the MS, VDB, HDB and NBM was reduced in SAP-PRh brains relative to controls. Indeed, the ANOVA revealed an overall significant effect of Group on ChAT-IR cell counts within the basal forebrain ($F_{1,11} = 5.16$, $P < 0.05$). There was also a significant effect of Region ($F_{1,33} = 14.85$, $P < 0.01$), but the Group–Region interaction was not significant ($F < 1$). Table 1 shows the mean number of ChAT-IR cells in each basal forebrain region for the two groups. Reductions were greatest in the HDB and NBM of the SAP-PRh group, with numbers of ChAT-IR cells reduced to approximately 69% and 74% of Control values, respectively. Although the Abercrombie correction factor was applied to cell counts before the statistical analyses, these results should still be interpreted with caution, as unbiased stereological techniques were not used during cell counting. As most neurons, however, measure 10–50 μm in diameter, it is unlikely that the present results have been influenced by multiple counts of the same cell, because sections used for the basal forebrain analyses were separated by a distance of 200 μm.

Analysis of PV-IR cells within the basal forebrain and PRh revealed no significant differences between the Control and SAP-PRh groups. The Group ($F < 1$) and Group × Region ($F < 1$) terms were both nonsignificant, suggesting that the lesion was selective for the cholinergic system. There was, however, a significant effect of Region ($F_{1,11} = 30.33$, $P < 0.001$). Table 1 shows the mean number of PV-IR cells in each basal forebrain region and PRh for the two groups.

Finally, Cresyl Violet staining of PRh revealed similar patterns in both groups (Fig. 1C and D), with no significant signs of nonspecific damage in the cortical infusion sites of the SAP-PRh group.

Object recognition

All animals explored the sample objects for the requisite 25 s in under 5 min on all trials and therefore no trials were excluded from the analysis on this basis. Analysis of the duration of the sample phase revealed no significant difference between the groups, as all animals explored the sample objects for the requisite 25 s in approximately the same amount of time on average ($t_{11} = 1.19$; means ± SEM: Control = 275.46 ± 12.87 s, SAP-PRh = 247.97 ± 18.17 s).

There was also no difference between groups in the total amount of time spent exploring the novel and familiar objects during the first minute of the choice phase ($t_{11} = 0.02$; means ± SEM: Control = 11.01 ± 1.45 s, SAP-PRh = 11.04 ± 1.53 s). SAP-PRh rats were, however, impaired relative to controls in object recognition and did not discriminate significantly between the novel and familiar objects in the first minute of the choice phase (see Fig. 2). Analysis of the discrimination ratio revealed a significant group effect ($t_{11} = 2.49$, $P = 0.03$). This effect was associated with increased familiar object exploration and reduced novel object exploration in the SAP-PRh group. The mean absolute exploration (± SEM) of novel and familiar objects in the first minute of the choice phase for each group was as follows: Control, Novel = 8.66 ± 1.29 s; Control, Familiar = 2.34 ± 0.34 s; SAP-PRh, Novel = 6.78 ± 1.59 s; SAP-PRh, Familiar = 4.26 ± 0.46 s).

Spatial alternation

The cross-maze performance of the groups did not differ (Fig. 3). There was no significant effect of Group ($F < 1$), Delay ($F < 1$), or the Group–Delay interaction ($F < 1$), indicating that SAP-PRh rats were as capable as Controls of utilizing allocentric spatial cues to alternate in this working memory task.

TABLE 1. ChAT-IR and PV-IR neurons in basal forebrain nuclei and PRh for each group

	Number of cells				
	MS	VDB	HDB	NBM	PRh
Control					
ChAT	444.95 ± 70.36	417.91 ± 65.56	359.3 ± 22.9	231.65 ± 20.21	N/A
PV	212.75 ± 38.72	135.32 ± 31.63	62.72 ± 20.67	70.38 ± 14.03	175 ± 13
SAP-PRh					
ChAT	366.3 ± 40.04	334.18 ± 17.74	248.51 ± 31.41	170.93 ± 21.88	N/A
PV	191.08 ± 25.08	146.58 ± 9.08	53.72 ± 13.69	70.46 ± 12.66	162.11 ± 30.11

Data are presented as mean ± SEM.

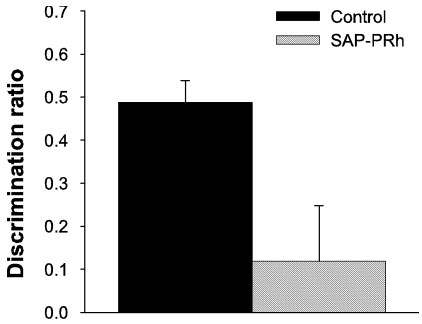

FIG. 2. Mean discrimination ratio (± SEM) for each group from the first minute of the choice phase in spontaneous object recognition.

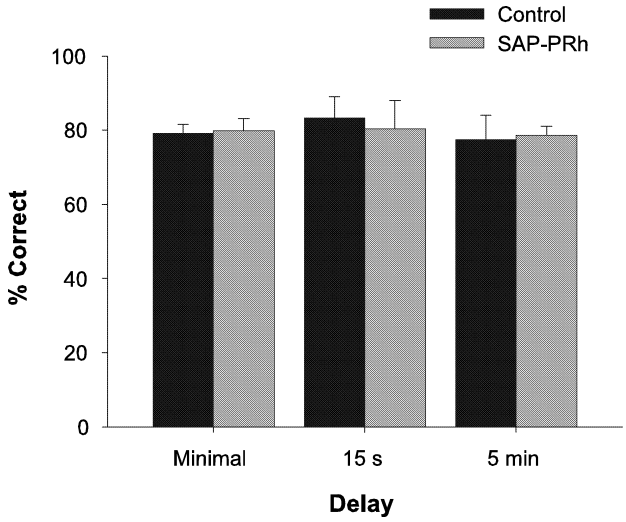

FIG. 3. Mean percentage correct (± SEM) for each group on cross-maze spatial alternation from each delay phase.

Discussion

The present results suggest an important role in object recognition memory for the cholinergic basal forebrain input to PRh. Injections of the cholinergic immunotoxin 192 IgG-saporin into PRh induced a selective reduction of the cholinergic input to this region. Staining for AChE, indicative of cholinergic fibres, was attenuated in PRh of the SAP-PRh group. The cholinergic innervation of the hippocampus was intact in the SAP-PRh group, as indicated by similar AChE staining patterns in the dorsal and ventral hippocampus of the SAP-PRh and Control groups. Moreover, numbers of ChAT-IR cells were reduced in those areas of the cholinergic basal forebrain (VDB/HDB, NBM) that project to PRh (Deacon *et al*., 1983; Woolf *et al*., 1984). Although these reductions were statistically significant, in magnitude they were only about 26–30% of Control values in the same basal forebrain regions. This significant, yet subtle, reduction in cholinergic cell bodies in the basal forebrain probably reflects the anatomical selectivity of the cholinergic lesion caused by injections of 192 IgG-saporin into PRh. This suggestion is consistent with intact cortical AChE staining outside of PRh, indicating that the majority of the corticopetal cholinergic neurons of the basal forebrain were not affected by the lesioning method employed. This is consistent with reports that these cortically projecting cholinergic neurons do not tend to collateralize, but rather innervate relatively discrete cortical areas (Bigl *et al*., 1982). Intracortical infusions of 192 IgG-saporin also did not have a substantial effect on the populations of PV-IR, presumably GABAergic, cells within the basal forebrain or PRh, and Nissl staining within PRh appeared to be normal in the brains of SAP-PRh rats.

Rats with this selective lesion were significantly impaired in object recognition memory but were unimpaired in a spatial memory task. This latter finding is consistent with a growing body of evidence suggesting that excitotoxic cell-body lesions of PRh do not affect the performance of spatial learning and memory tasks that depend on intact hippocampal functioning (Gaffan, 1994; Ennaceur *et al*., 1996; Bussey *et al*., 1999, 2000; Winters *et al*., 2004a; but cf. Liu & Bilkey, 1998a, b). These same lesions do, however, impair object recognition memory (Bussey *et al*., 1999; Winters *et al*., 2004a). This pattern of results may be related to the distinct perceptual demands of the object recognition and spatial memory tasks. PRh is now believed to mediate the representation of complex visual stimuli (Murray & Bussey, 1999; Bussey & Saksida, 2002), and several recent studies have implicated PRh in perceptual and complex visual discrimination tasks (Buckley & Gaffan, 1997, 1998; Buckley *et al*., 2001; Eacott *et al*., 2001; Bussey *et al*., 2002a,b, 2003). It is likely that the complex 'junk' objects used for the object recognition task place a greater demand on the perceptual functions of PRh than the simpler stimuli used for navigation in the cross-maze task. Perhaps if the distal cues commonly used in spatial navigation tasks were made more visually complex, PRh and its cholinergic afferents might be recruited into the circuitry required for successful performance (Bussey & Aggleton, 2002; Aggleton *et al*., 2004).

Despite the lack of effect on spatial memory, SAP-PRh rats were impaired relative to the sham-operated group on the object recognition task. This result is consistent with recent reports of object recognition deficits in rats receiving intra-PRh infusions of scopolamine (Warburton *et al*., 2003; Abe *et al*., 2004) and has strong implications for the role of cholinergic basal forebrain projections in object recognition memory. The present result suggests that cholinergic projections from the basal forebrain, which were targeted by 192 IgG-saporin infused into PRh, are necessary for the successful recognition of a familiar object. Although it is clear from past studies that blockade of muscarinic cholinergic receptors in PRh can disrupt object recognition (Tang *et al*., 1997; Warburton *et al*., 2003; Abe *et al*., 2004), the source of ACh with which scopolamine presumably interfered in these studies remained uncertain. We have now provided evidence that cholinergic input arising in nuclei of the basal forebrain contribute crucially to object recognition processes mediated by PRh. The present finding suggests that blockade of cortical receptors in regions innervated by these projections is probably the primary cause of object recognition deficits observed following intra-PRh infusions of scopolamine. Moreover, the present results extend those earlier reports by showing that a similar deficit to that caused by acute muscarinic receptor blockade in PRh can be produced by chronic removal of the source of cholinergic innervation of PRh. It is interesting that, unlike in the hippocampus (Parent & Baxter, 2004), the behavioural effects of selective ablation of cholinergic projections are similar to those resulting from transient muscarinic receptor blockade in PRh. Inconsistent findings from hippocampal manipulations may indicate the use of alternative, hippocampal ACh-independent, strategies to solve the spatial tasks used (Parent & Baxter, 2004) or the possible influence of compensatory changes occurring in response to permanent lesions. It is possible that the simplicity of the spontaneous object recognition task protects it from the former and that cholinergic basal forebrain innervation of PRh is, indeed, necessary for successful object recognition memory, consistent with previous reports using intra-PRh infusions of scopolamine (Tang *et al*., 1997; Warburton *et al*., 2003; Abe *et al*., 2004).

An alternative explanation for the impairment observed following intra-PRh 192 IgG-saporin could be that the resulting partial lesion of

the cholinergic basal forebrain was merely large enough to surpass the threshold of basal forebrain damage required to uncover an object recognition deficit. It has been suggested for spatial memory tasks that such a threshold relationship exists, whereby a high degree of cholinergic basal forebrain damage can occur before spatial working memory deficits are observed (Wrenn *et al*., 1999). This is consistent with several studies demonstrating intact spatial memory performance following more selective cholinergic pathway lesions and the emergence of deficits only when the cholinergic basal forebrain damage is nearly complete, usually resulting from relatively high doses of intracerebroventricular infusions of 192 IgG-saporin (Berger-Sweeney *et al*., 1994; Baxter *et al*., 1995, 1996; Leanza *et al*., 1995; Waite *et al*., 1995; Walsh *et al*., 1995; Baxter & Gallagher, 1996). Such findings suggest that the cholinergic basal forebrain acts rather diffusely to mediate performance in spatial memory tasks, an assertion consistent with the anatomically diffuse projections of cholinergic basal forebrain nuclei. Indeed, it is possible that the corticopetal cholinergic basal forebrain projections act together to regulate neuronal excitability within the cortex and that this function is crucial for certain spatial memory tasks. It is therefore difficult to conclude with complete certainty that the object recognition impairment in the present study was due to removal of cholinergic input to PRh *per se*. It is not known whether a similar reduction of input to some other cortical region would have the same behavioural impact purely by disrupting cholinergic basal forebrain function to the same extent as the present lesion.

The present results, however, consistent with other findings demonstrating important differences between the circuitry involved in spatial and object recognition tasks (Bussey *et al*., 2000; Bussey & Aggleton, 2002; Winters *et al*., 2004a), suggest that such a clear threshold relationship may not exist for object recognition memory. In the present study, a very subtle (an overall reduction of just 23% of Control levels of ChAT-IR cells in the regions counted) but selective reduction in cortical cholinergic input was sufficient to demonstrate significant object recognition impairment. Although it is possible that the threshold for basal forebrain damage required in object recognition is much lower than for spatial memory tasks, it seems more likely that the specific projections to PRh lesioned in the present study play a direct role in object recognition memory. In the future, inclusion of a control lesion condition in which some other cortical region is injected with 192 IgG-saporin could help to resolve this issue. However, the fact that fimbria–fornix transection or large excitotoxic lesions of the hippocampus fail to impair object recognition (Ennaceur *et al*., 1996; Bussey *et al*., 2000; Winters *et al*., 2004a; Forwood *et al*., 2005) argues somewhat against the threshold hypothesis, as these lesions would be expected to cause substantial basal forebrain damage as a result of retrograde degeneration (Sofroniew *et al*., 1986, 1987; Tuszynski *et al*., 1990).

The question remains as to what exact function ACh plays in PRh to facilitate object recognition memory. Cortical ACh has a well-established role in certain attentional processes (Jones & Higgins, 1995; McGaughy *et al*., 1996, 2002; Sarter & Bruno, 1997; Sarter *et al*., 2001), and electrophysiological findings suggest that cholinergic application to cortical pyramidal cells can fine-tune the receptive fields of these cells (Krnjevic & Phillis, 1963; Sillito & Kemp, 1983; Murphy & Sillito, 1991; Rasmusson, 2000). Such evidence suggests that ACh within PRh might contribute to object recognition through perceptual or attentional processes to facilitate encoding and acquisition. Indeed, there is evidence from human studies that increased cortical ACh can enhance the selectivity of perceptual processing in extrastriate cortex during encoding in a visual working memory task (Furey *et al*., 1997, 2000b). Furthermore, the multitude of studies reporting object recognition impairment following scopolamine administration before the acquisition or sampling phase (Aigner & Mishkin, 1986; Huston & Aggleton, 1987; Aigner *et al*., 1991b; Tang *et al*., 1997; Warburton *et al*., 2003) is consistent with a role for ACh in encoding.

There is accumulating evidence for ACh involvement in certain forms of cortical synaptic plasticity (Segal & Auerbach, 1997; Rasmusson, 2000). Brown and colleagues, in particular, have demonstrated a potentially crucial role for ACh in object recognition-related long-term depression in PRh (Massey *et al*., 2001; Bashir, 2003; Warburton *et al*., 2003). Specifically, it has been shown that bath application of carbachol to activate cholinergic receptors *in vitro* in PRh induces a long-lasting depression of synaptic transmission that requires muscarinic M1 receptor activation (Massey *et al*., 2001). Moreover, the induction of this long-lasting depression is independent of *N*-methyl-D-aspartic acid receptor activation but requires protein synthesis. Extending these findings, Warburton *et al*. (2003) have reported that intra-PRh scopolamine, which impairs object recognition, also disrupts the normal PRh neuronal response decrement to familiar visual stimuli and blocks the induction of long-term depression of synaptic transmission in PRh slices. These results provide strong and compelling evidence for a role of ACh in PRh in mnemonic, and not just perceptual or encoding, aspects of object recognition. The effect of muscarinic receptor blockade on synaptic plasticity is particularly suggestive of a possible role for ACh in early consolidation and storage mechanisms within PRh. However, the results of the few published attempts to assess effects of postacquisition scopolamine administration are less clear, as some have reported no effect of systemic postsample injections (Warburton *et al*., 2003), whereas others report object recognition impairment following postsample intra-PRh infusions (Abe *et al*., 2004). Thus, the role of PRh ACh in object recognition acquisition vs. consolidation processes remains uncertain. Regardless of its specific function, the present findings, taken with past results from intra-PRh scopolamine, indicate that cholinergic innervation of PRh is crucial for successful object recognition memory.

In summary, the present study has shown, consistent with recent findings with intra-PRh scopolamine infusions, that cholinergic activity within PRh is important for object recognition memory in rats. Selective lesions of the cholinergic projections from the basal forebrain to PRh impaired spontaneous object recognition performance, but spared allocentric spatial working memory, indicating that the impairment was not a global memory disruption. The cholinergic basal forebrain input to PRh may facilitate object encoding and/or consolidation processes involved in storage of the memory trace shortly following acquisition.

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Abbreviations

ACh, acetylcholine; AChE, acetylcholinesterase; ChAT, choline acetyltransferase; GABA, gamma-amino-butyric acid; HDB, horizontal limb nucleus of the diagonal band; IR, immunoreactive; MS, medial septum; NBM, nucleus basalis magnocellularis; PRh, perirhinal cortex; PV, parvalbumin; SAP-PRh, group receiving 192 IgG-saporin injections into perirhinal cortex; VDB, vertical limb nucleus of the diagonal band.

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