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RESEARCH ARTICLE

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Intact negative patterning in rats with fornix or combined perirhinal and postrhinal cortex lesions

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Abstract It has been proposed that the hippocampal formation is necessary for the acquisition of tasks that require the use of configural representations for their solution, including spatial learning and negative patterning. Tests of this influential view have, however, yielded conflicting results. For example fornix or hippocampal lesions, which reliably impair spatial learning, do not reliably impair negative patterning. A problem in interpreting these results has been the lack of controls for factors such as over-responding, excitatory effects of reward, and the possibility of non-configural solutions. At the same time, other studies have pointed to a role in configural learning for parahippocampal regions such as the perirhinal cortex. The present experiments controlled for the above factors and revealed that neither lesions of the fornix nor of the perirhinal/postrhinal cortex in the rat had any effect on negative patterning, although subsequent tests of object and spatial memory demonstrated the functional efficacy of the lesions.

Key words Hippocampus · Negative patterning · Object discrimination · Autoshaping · Inferotemporal cortex

Introduction

The issue of how best to characterise the role of the hippocampus in learning and memory continues to generate much research and debate. One challenge has been how to reconcile the episodic memory deficits observed following hippocampal damage in humans with spatial memory deficits following hippocampal damage in rats. To address this and other issues Sutherland and Rudy (1989) introduced an ingenious idea. According to their "configural association theory", the hippocampus processes and stores configural representations of stimuli. It was suggested that compromising this function can lead to deficits in spatial learning in rats, and amnesia in humans. In response to apparently contradictory evidence (Davidson et al. 1993; Gallagher and Holland 1992; Saunders and Weiskrantz 1989; Whishaw and Tomie 1991), these authors modified their theory to propose that the hippocampus mediates configural learning by acting upon stimulus representations stored in the neocortex (Rudy and Sutherland 1995). The revised form of the theory, like the original version, views spatial learning as a special case of configural learning, but unlike the original view predicts that hippocampal system damage should impair the acquisition of only certain configural tasks. Such tasks include transverse patterning (a pairwise concurrent discrimination task: A+ v B–; B+ v C–; C+ v A–) and negative patterning (in which presentation of either of two stimuli alone is reinforced, but conjoint presentation is not: A+, B+, AB–).

Even in its revised form, however, the configural association theory of Rudy and Sutherland (1995) has encountered contradictory evidence. Particularly problematic is a recent study showing that fornix lesions, which disrupt many of the inputs to and outputs from the hippocampus and thus typically mimic the effects of hippocampal lesions, did not disrupt acquisition of the transverse patterning task. Instead, these lesions *facilitated* configural learning in this paradigm (Bussey et al. 1998). Importantly, these same animals were severely impaired in spatial learning in the T-maze and Morris water maze. Configural association theory does not explain how, if spatial learning is a special case of configural learning, the same manipulation that consistently impairs spatial learning can spare, or indeed facilitate, configural learning.

In light of the foregoing issues, the present study sought to further our understanding of the brain regions involved in configural learning by investigating the

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effects on negative patterning of lesions of the fornix and of the perirhinal and postrhinal cortices.

Fornix lesions

The negative patterning task is the canonical example of a configural task, yet the effects of hippocampal or fornix lesions on this task have been inconsistent. Davidson et al. (1993) pointed out that previous apparent impairments in negative patterning following hippocampal damage may have been due the lesioned animals' increased response rates. Using a procedure that controlled for response rates, these authors showed that negative patterning was not disrupted by hippocampal lesions. Importantly, these authors administered appropriate transfer tests to provide evidence that these animals were not using non-configural strategies to solve the problem. McDonald et al. (1997), however, argued that Davidson et al. (1993) had not sufficiently controlled for factors related to the putative excitatory properties of food reward delivered during a trial, or the use of reward as a cue for the availability of further reward during a trial. Using a procedure designed to control for such effects, McDonald et al. (1997) reported that hippocampal, but not fornix lesions, impaired negative patterning. McDonald et al. (1997), however, unlike Davidson et al. (1993), did not control for response rates, nor did they administer appropriate transfer tests to provide evidence that animals were not using non-configural strategies to solve the problem. Thus the effect of such lesions on negative patterning remains unclear.

In the present study we address these issues using a negative patterning paradigm developed by Redhead and Pearce (1995). This paradigm has the following important features which control for all of the above factors:

1. Conditioning is measured by computing the difference between magazine activity during the 10-s conditioned stimulus (CS) and activity during the 10 s immediately prior to the CS. This measure thus controls for high response rates by reflecting activity relative to baseline levels. Furthermore, the task is Pavlovian rather than instrumental; thus animals receive reward on A+ and B+ trials irrespective of whether a response is emitted.
2. Reward is delivered at the end of the 10-s CS, thus controlling for excitatory influences of food reward on responding, and eliminating the use of reward as a cue for the availability of further reward during a trial.
3. Transfer tests can be administered following acquisition to test whether animals use non-configural strategies such as intensity or numerosity of cues. To these we added extinction trials as an additional test of whether lesioned animals would show an inability to withhold responding.

Our initial investigations using this paradigm, outlined in the present article, focused on the effects of lesions of the fornix. The fornix was targeted for several reasons. Firstly, fornix lesions lead to hippocampal dysfunction without the risk of direct damage to parahippocampal regions. This is important as there is reason to believe that these regions may themselves be important for configural learning (see below). Secondly, even when direct parahippocampal damage is avoided, transneuronal degeneration in these regions can occur following hippocampal lesions, a problem that can be ameliorated by using fornix lesions. Finally, fornix lesions are appropriate for testing configural association theory as this theory assumes that spatial learning is a special case of configural learning, and it is well established that fornix lesions can lead to deficits as severe as those obtained following hippocampal lesions.

Perirhinal and postrhinal cortex lesions

If the hippocampus is not generally necessary for the acquisition of configural tasks, as suggested by Bussey et al. (1998), are there other regions within the temporal lobe that might be involved in this type of learning? There are several reasons to suppose that such a region might lie within the perirhinal/postrhinal cortex. Buckley and Gaffan (1998a), for example, report that perirhinal cortex lesions in the monkey disrupt configural learning. Furthermore, Eichenbaum and Bunsey (1995) have suggested that parahippocampal regions "fuse" stimuli into configural-like representations, and Gluck and Myers (1995) suggest that parahippocampal cortical regions are required for "redundancy compression", a concept not unlike that of the formation of configural representations described by Sutherland and Rudy (1989). Finally, a recent neural network model of perirhinal cortex (Saksida and Bussey 1998), based on the anatomical and electrophysiological properties of this region, predicts that lesions including perirhinal cortex should disrupt configural learning involving sufficiently complex visual stimuli (as shown by Buckley and Gaffan 1998a), but not necessarily with simple stimuli, the configural representations of which are stored in visual regions upstream from perirhinal cortex (see also Murray and Bussey 1999). The present study tests part of this prediction by examining configural learning with simple stimuli.

Following testing on negative patterning and transfer tests, lesioned animals were tested on a Pavlovian autoshaping task to determine the effects of these lesions on simple conditioning, and, in order to test the functional efficacy of the lesions, on tests of spontaneous object recognition, spatial memory and spontaneous locomotor activity. These tests have been shown to be differentially affected by lesions of the fornix and perirhinal/postrhinal cortex (Aggleton et al. 1997; Bussey et al. 1999, 2000).

Experiment 1: negative patterning

Materials and methods

Subjects

Forty-two male rats of the pigmented DA strain (Bantin and Kingman, UK) weighing 220–250 g were housed in pairs in a temperature-controlled room on a 14 h light/10 h dark cycle. Animals were provided with free access to water and by means of a restricted feeding regimen were maintained throughout the experiments at or above 85% of their free feeding weight. "Principles of laboratory animal care" (NIH publication, 1985) were followed.

Surgical and histological methods

Rats were divided into four groups according to surgical treatment: fornix-lesioned (FNX, *n*=15), perirhinal/postrhinal cortex-lesioned (PPRH, *n*=14) and surgical controls (CONTfnx, *n*=8; CONTpprh, *n*=5).

Fornix lesions. Each animal was anaesthetised by intraperitoneal injection of pentobarbitone sodium (Sagatal; Rhone Merieux, UK) at a dose of 60 mg/kg. The animal was then placed in a stereotaxic frame (David Kopf Instruments, USA) with the nose bar at +5.0 and the scalp retracted to expose the skull. Radiofrequency lesions of the fornix were made using an RFG4-A Lesion Maker (Radionics, UK). The electrode (0.3 mm tip length, 0.25 mm diameter) was lowered vertically and at each site the temperature of the tip was raised to 75°C for 60 s. The coordinates for lesions of the fornix were (AP, LM from ear-bar zero, DV from top of cortex): (1) AP +5.3, LM ±0.7, DV –3.7; and (2) AP +5.3, LM ±1.7, DV –3.8. Following surgery the skin was sutured, animals were given a 0.01 ml intraperitoneal injection of analgesic (Temgesic; Reckett and Colman, UK), and an antibiotic powder (Acramide, Dales Pharmaceuticals, UK) was applied. Animals of the CONTfnx group received the same surgical procedure with the exception that the temperature of the tip of the electrode was not raised.

Perirhinal/postrhinal cortex lesions. All animals were deeply anaesthetised by intraperitoneal injection (60 mg/kg) of pentobarbitone sodium and then placed in a stereotaxic headholder with the nose bar at +5.0. The scalp was then cut and retracted to expose the skull. For the perirhinal/postrhinal cortex lesion, injections of 0.2 µl 0.09 M *N*-methyl-D-aspartic acid (Sigma Chemical Company, UK) dissolved in phosphate buffer (pH 7.2) were made through a 1-µl Hamilton syringe into five sites in each hemisphere. Each injection was made gradually over a 5-min period and the needle was left in situ for a further 4 min before being withdrawn. The stereotaxic co-ordinates relative to ear-bar zero were: AP +3.9, LM ±5.9, DV 2.0; AP +2.4, LM ±6.1, DV +1.6; AP +0.6, LM ±6.2, DV +2.5; AP –0.8, LM ±6.2, DV±2.7; and AP –0.8, LM ±6.2, DV +4.3. Animals of the CONTpprh group received exactly the same initial surgery, i.e. craniotomy but no injections were made. At the completion of all surgeries the skin was sutured, animals were given an 0.01 ml intraperitoneal injection of analgesic (Temgesic; Reckett and Colman, UK), and an antibiotic powder (Acramide; Dales Pharmaceuticals, UK) was applied.

Histological methods. Following completion of the experiment, the animals were anaesthetised with intraperitoneal injection of pentobarbitone sodium (Euthatal, Rhone Merieux) and perfused transcardially with saline followed by 10% formol-saline. The brain was removed and postfixed in formol-saline for a minimum of 2 h before being transferred into 20% sucrose in 0.2 M phosphate buffer and left overnight. Coronal sections were cut at 60 µm on a freezing microtome and stained with cresyl violet Nissl stain.

Negative patterning methods

Apparatus. Four identical operant chambers were used (24.5× 23.0×20.0 cm), each housed in a separate sound-attenuating box equipped with a fan for ventilation and masking of noise. The ceiling was made of opaque Perspex, the front wall was made of clear Perspex and the remaining three walls were made of aluminium. A 5-W speaker located on the back wall of the chamber was used to present a 10-s, 2-kHz, 90-dB tone stimulus, and a 5-W speaker on the front wall was used to present a 10-s, 10-Hz, 65-dB clicker stimulus. A 240-V, 60-W strip light located above the opaque Perspex ceiling was operated via a thyristor circuit except when extinguished to produce a 10-s "darkness" stimulus. A pellet dispenser (Campden Inst., UK) situated outside of the chamber was used to dispense food pellets (45 mg; Campden Inst.) into a food magazine (5×6 cm) located centrally within the wall of the chamber. Animals gained access to the magazine via a hinged Perspex flap. Surrounding the food magazine was a 10-mm deep rectangular frame containing three pairs of photocells (infra-red emitters and photo-diode sensors) that detected entries into the magazine. The apparatus was controlled and monitored by an Acorn microcomputer, using programs written in BBC BASIC and "Arachnid" on-line control procedures (Paul Fray, UK).

Behavioural procedures. Rats were pretrained to collect food pellets from the magazine during two 30-min sessions in which food pellets were delivered according to a variable-time (VT) 60-s schedule. During the first session the magazine flap was taped open for easy access; during the second session animals had to push the panel open with their snouts to gain access to the pellets.

For negative patterning training, animals received 50 sessions of discrimination training in each of which there were 24 trials presented according to a variable interval (VI) 120-s schedule (range 60–180 s). In every session there were four presentations of the 10-s tone (A+) and of the 10-s darkness (B+) stimulus. Immediately upon offset of a stimulus a single food pellet (45 mg) was delivered into the magazine. The task was thus "Pavlovian" in that delivery of reward was not contingent on the animal's response. Also presented were 16 trials in which the tone and darkness were presented simultaneously for 10 s. These compound stimuli (AB–) were not followed by pellet delivery. The order of trial presentation was determined pseudorandomly, with the constraint that no more than two trials of the same type could occur consecutively. Throughout the experiment the measure of conditioning was magazine activity: the time spent in the food magazine during the 10-s CS, minus the time spent in the magazine during the 10 s just preceding the onset of the CS. This measure was used to control for possible differences in rates of responding.

Following negative patterning training, rats were divided into two approximately equal groups. One group was given transfer tests (group sizes following histological analysis: CONT *n*=7, FNX *n*=5, PPRH *n*=6), and one group was given an extinction test (group sizes following histological analysis: CONT *n*=5, FNX *n*=4, PPRH *n*=6).

Although the prevailing view is that the solution of the negative patterning task requires some form of configural learning (Pearce 1987, 1994; Wagner and Rescorla 1972), it is conceivable that animals could learn this discrimination using non-configural cues such as "intensity" or "numerosity". For example, rats could learn the general rule that one stimulus signals reward, but that two stimuli signal non-reward. We used transfer tests to determine whether this was the case. Throughout these transfer tests stimuli were presented according to the same VT 120-s schedule (range 60–180 s) as was used during negative patterning training. First, animals were given three sessions, each consisting of 24 presentations of a 10-s clicker stimulus (C+), which was always followed immediately by the delivery of a food pellet. A single session was then given in which clicker presentation was combined with the negative patterning problem (A+, B+, AB–, C+; the session consisted of 4 trials of A+, 4 of B+, 12 of AB– and 4 of C+). This was followed by a critical transfer test session in which the clicker was presented in compound with the tone and darkness stimuli (A+, B+, AB–, C+, AC–, BC–; the session consisted of 4 trials of

each type). If rats had learned to solve the negative patterning problem using numerosity or intensity, then this learning would be expected to transfer to the new AC– and BC– compound stimuli and rats would suppress responding on these trials as they did on AB– trials. If, however, rats had learned a configural solution particular to the stimuli used in the negative patterning discrimination, no transfer would be expected and they would respond at a higher rate to AC– and BC– than to AB–. To ensure validity of this test, however, it is important to show that rats were able to suppress responding in the presence of compounds involving the clicker. Thus, one session was given in which rats were presented with 5 trial types: A+, B+, AB-, C+, ABC– (the session consisted of 4 trials of A+, 4 of B+, 6 of AB–, 4 of C+ and 6 of ABC–). As suppression to AB– should generalise more to ABC– than to A+, B+ or C+, we expected to see suppression to ABC–, showing that rats can suppress in the presence of the clicker stimulus. This result is predicted by the configural associative theory of Pearce (1994); thus this last discrimination provided an independent test of this theory that to our knowledge had not been attempted previously.

For the extinction trials, animals first received one session in which there were 24 trials presented according to a VI 120-s schedule (range 60–180 s). In this session there were twelve presentations of the 10-s tone (A+) and of the 10-s darkness (B+) stimulus. Immediately upon offset of a stimulus a single food pellet was delivered into the magazine. The following day a test session, consisting of 24 trials, was given. In this session the first 12 trials were the same as in the previous session, with six presentations of the 10-s tone (A+) and of the 10-s darkness (B+) stimulus. The final 12 trials were extinction trials, in which all task parameters were the same with the exception that no food pellets were delivered.

Autoshaping methods

Apparatus. The apparatus is a modification of an apparatus described elsewhere (Bussey et al. 1994, 1997). It consisted of a testing chamber and video display unit (VDU) housed within a wooden sound-attenuating box, fitted with a fan for ventilation and masking of extraneous noise. The inner chamber measured 48×30×30 cm, and consisted of a metal frame, clear Perspex walls and an aluminium floor. A 3-W houselight was attached to the ceiling of the chamber. Located centrally in front of the VDU was a food magazine attached to a pellet dispenser situated outside of the sound-attenuating box. The magazine was fitted with photocells that detected when a rat entered the magazine. Sets of photocells were also located to the left and right of the magazine, directly in front of the VDU screen. These photocells detected approaches to the left and right sides of the VDU screen, where stimuli were presented. A pressure-sensitive floor panel was located centrally at the other end of the chamber. The stimuli used were white vertical rectangles, 10 cm wide by 28 cm high, displayed on the left and right of the VDU screen. The apparatus was controlled and monitored by an Acorn microcomputer, using programs written in BBC BASIC and using "Arachnid" on-line control procedures.

Behavioural procedures. Rats were initially given one session in which they were allowed to habituate to the testing chamber and to collect pellets from the magazine. The session consisted of 50 trials in which pellets were delivered three at a time into the magazine on a VI 40-s schedule. On the 2nd day of pretraining the rats received the same training with the exception that delivery of pellets was contingent on the rat being located on the rear floor panel following the intertrial interval. On the 3rd day of pretraining rats again received the same training with the exception that only one pellet was delivered on each trial. Additional sessions were given as necessary until the latency to collect pellets was stable (usually only one additional session was needed).

On the day following pretraining, rats were trained to associate a stimulus with reward. The two stimuli used were identical vertical white bars, presented on the left or right of the VDU screen. Stimuli were presented for 10 s. One of the stimuli was designated the CS+ and signalled the delivery of a food pellet immediately following stimulus offset; the other, designated the CS–, was never followed by food delivery. The task is thus "Pavlovian" in that delivery of reward was not contingent on the rat's response. As rats learn to associate the CS+ with reward, they approach the CS+ with increasing frequency and decreasing latency (Bussey et al. 1997). The left stimulus was designated the CS+ for half of the animals in each of the experimental groups; for the other half the right stimulus was designated the CS+. Both the CS+ and CS– were presented on a VI 40-s schedule, and training consisted of 100 presentations of both the CS+ and CS–. When the rat crossed through the photocells in front of a stimulus, it was scored as an approach to that stimulus, and no further approaches were scored during that stimulus presentation.

Presentation of stimuli and collection of response measures were subjected to the following restrictions: (1) stimuli were not presented until the rat was centrally located at the rear of the chamber (this eliminated chance approaches to the stimuli, provided all rats on every trial with an equivalent opportunity for stimulus sampling, and allowed for the accurate calculation of approach latencies based on the time taken for rats to approach the stimuli starting from the same position in the test chamber on each trial), (2) the minimum time between presentations of the CS+ and CS– was 10 s and (3) the maximum number of consecutive presentations of either the CS+ or the CS– was two.

Several performance measures were calculated: (1) the number of approaches to the CS+ per ten presentations, (2) the number of approaches to the CS– per ten presentations, (3) the number of entries into the magazine during the CS+ per ten presentations, (4) the number of entries into the magazine during the CS– per ten presentations, (5) the mean latency to approach the CS+ (to the nearest 1/100 of a second) and (6) the mean latency to approach the CS– (to the nearest 1/100 of a second).

Results

Histological analysis

Perirhinal/postrhinal cortex lesions. One of the 14 animals with a perirhinal/postrhinal cortex lesion was rejected as the cortical damage extended dorsally into V1 while in another the lesions were unusually small. In the remaining 12 perirhinal/postrhinal cortex lesion cases the neurotoxin consistently removed almost all of the perirhinal and postrhinal cortex starting from the midlevel of the amygdala to close to the very caudal limit of the postrhinal cortex (see Fig. 1).

The rostral limit of the lesions was at or in front of the midlevel of the amygdala, and corresponded to the rostral limit of the perirhinal cortex as depicted by Swanson (1992). Caudally the lesions always extended to within the last millimetre of the postrhinal cortex and in four cases the entire postrhinal cortex was removed bilaterally. Within the extent of the lesions there was no evidence of neuronal sparing, although in three cases the lesions did spare the most dorsal edge of area 36 at a few, restricted AP levels. At the more rostral levels the lesions consistently encroached ventrally to include approximately two-thirds of the piriform cortex (see Fig. 1), and in ten cases there was partial damage to the lateral nucleus of the amygdala (bilateral in one of these case). More caudally, the lesions consistently involved the lateral

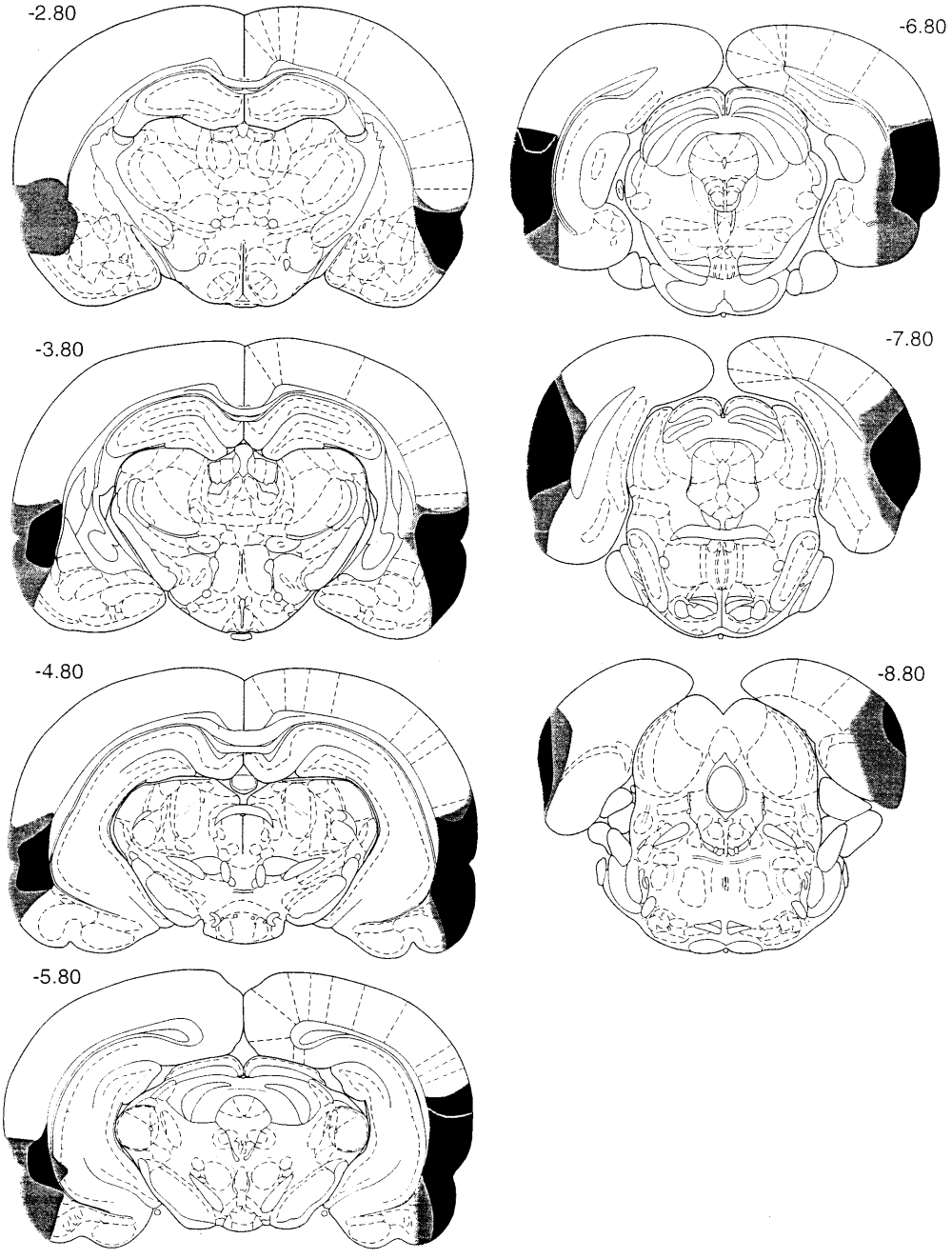

Fig. 1 Coronal sections illustrating the extent of the largest (*grey*) and smallest (*black*) lesions of the perirhinal/postrhinal cortex. The *numbers* correspond to the approximate position from bregma (Paxinos and Watson 1997)

portions of the lateral entorhinal cortex, but always spared the medial entorhinal cortex. The lesions were variable in their dorsal extent. Area TE was partially involved in all PPRH animals, and in eight cases the lesions also included restricted parts of the auditory association cortex immediately dorsal to TE (unilateral in six cases, bilateral in two cases). Although the neurotoxic damage extended throughout all layers of the rhinal sulcus, except in the case with the smallest lesion (see Fig. 1), the hippocampus showed remarkably limited damage. A very restricted bilateral patch of cell loss was observed in CA1 in five cases. In three others this cell loss was only unilateral, and in two others the only hippocampal cell loss was in the lateral margin of the retrohippocampal region. In two cases there was appreciable, unilateral damage to the parietal cortex where the injection needles passed through the cortex.

Fornix lesions. Five animals in which there was considerable sparing of the fimbria/fornix were rejected and their data discarded. In the remaining ten animals the fornix lesions produced extensive bilateral damage to the tract. The median extent of damage involved 90% of the tract, with the sparing typically occurring at the most lateral tips of the tract. While the largest lesions severed the entire fimbria/fornix, the smallest lesions spared the most lateral 30% of the tract (see Fig. 2). In one case the tract lesion extended caudally to involve the head of the

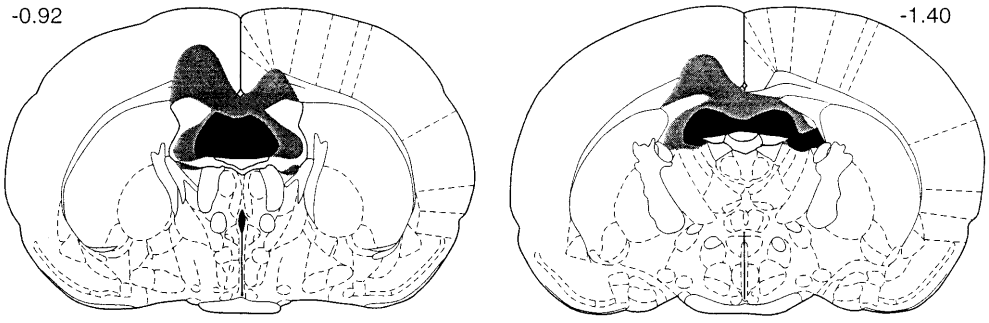

Fig. 2 Coronal sections illustrating the extent of the largest (*grey*) and smallest (*black*) lesions of the fornix. The *numbers* correspond to the approximate position from bregma (Paxinos and Watson 1997)

hippocampus, and in all cases the lesions extended ventrally to just touch the dorsalmost margin of the anterior thalamic nuclei. In six cases the cingulum bundle was cut bilaterally and there was damage to the adjacent cingulate cortex.

Following histological analysis one of the control animals was discarded as it had suffered an infection, leaving the following group sizes: CONT n=12, FNX n=10, PPRH n=12.

Negative patterning

Acquisition One animal in the FNX group did not reliably retrieve pellets from the magazine during training. This animal was therefore removed from negative patterning training and instead was given additional magazine training. This animal was thus able to rejoin the cohort in subsequent phases of the study (autoshaping onwards). Group sizes for this phase of the experiment were thus: CONT n=12, FNX n=9, PPRH n=12.

As shown in Fig. 3, there were no significant differences between any of the CONT, FNX or PPRH groups in their ability to solve the negative patterning problem. Thus there was no significant main effect of Group, $F(2,30)<1$, and no Group×Stimulus interaction, $F(2,30)=1.84$. There was a main effect of Block, $F(24,720)=24.9$, $P<0.0001$, and a significant Block by Stimulus interaction, $F(24,720)=53.9$, $P<0.0001$, but no significant Group×Block, $F(48,720)<1$, or Group×Block×Stimulus interaction, $F(48,720)=1.14$. There was, however, a highly significant main effect of Stimulus, $F(1,30)=209.9$, $P<0.0001$, showing that animals were able to discriminate the rewarded ELEMENTS from the non-rewarded COMPOUND. Final performance levels of the three groups are compared in Fig. 4.

Transfer tests. Group sizes for this phase of the experiment were: CONT n=7, FNX n=5, PPRH n=6. Data from the A+, B+, AB–, C+, AC–, B- discrimination are presented in Fig. 5. Magazine activity during A+ and B+ was averaged, as was magazine activity during compounds containing the clicker, AC– and BC–. Analysis of variance revealed no significant effect of Group, $F(2,15)<1$, and no significant Group×Stimulus interaction, $F(6,45)<1$. There was, however, a highly significant main effect of Stimulus, $F(3,45)=34.0$, $P<0.0001$. Newman-Keuls *post hoc* comparisons revealed that although animals continued to suppress responding during AB– relative to A+/B+, C+ and AC–/BC– (all $P<0.01$), there was no significant difference between magazine activity during A+/B+ and AC–/BC–, or, most importantly, between C+ and AC–/BC–. Thus this transfer test provides no evidence that animals solved the negative patterning discrimination using intensity or numerosity. To ensure validity of this test, however, is important to show that rats were able to suppress responding in the presence of compounds involving the clicker. In order to test this, an A+, B+, AB–, C+, ABC– discrimination was given to determine whether these rats were able to suppress responding to ABC– relative to C+. The critical comparison between magazine activity during C+ and ABC– revealed that rats responded significantly more during C+ than during ABC–, $F(1,15)=19.63$, $P<0.001$, showing that the rats could suppress responding to a compound containing C+. Again there were no significant differences between the three groups, $F(2,15)<1$, and no significant Group×Stimulus interaction, $F(2,15)<1$. Analysis of simple effects revealed a significant effect of stimulus for each of the CONT ($P<0.01$), FNX ($P<0.05$) and PPRH ($P<0.05$) groups. Thus each of these groups responded more to C+ than to ABC–. This final test provided independent support for the configural associative learning theory of Pearce (1994).

Extinction. Group sizes for this phase of the experiment were: CONT n=5, FNX n=4, PPRH n=6. As shown in Fig. 6, there were no significant differences between any of the CONT, FNX or PPRH groups in the rate of extinction to the positively reinforced elements. The session consisted of two blocks of six trials each. During the first, "baseline" block presentations of A and B were followed by the delivery of a food pellet and during the second, "extinction" block no pellets were delivered. Analysis of variance with Group as the between-subjects factor and Block and Trial as within-subjects factors revealed no significant effect of Group, $F(2,12)=1.44$, $P=0.28$, and no significant interactions involving the Group factor. To increase the chances of observing group differences, separate ANOVAs were performed on the two blocks separately. Analysis of the Baseline block revealed no significant group differences, $F(2,12)=1.3$, and no Group×Trial interaction, $F(10,60)<1$. Analysis of the Extinction block similarly revealed no group differences,

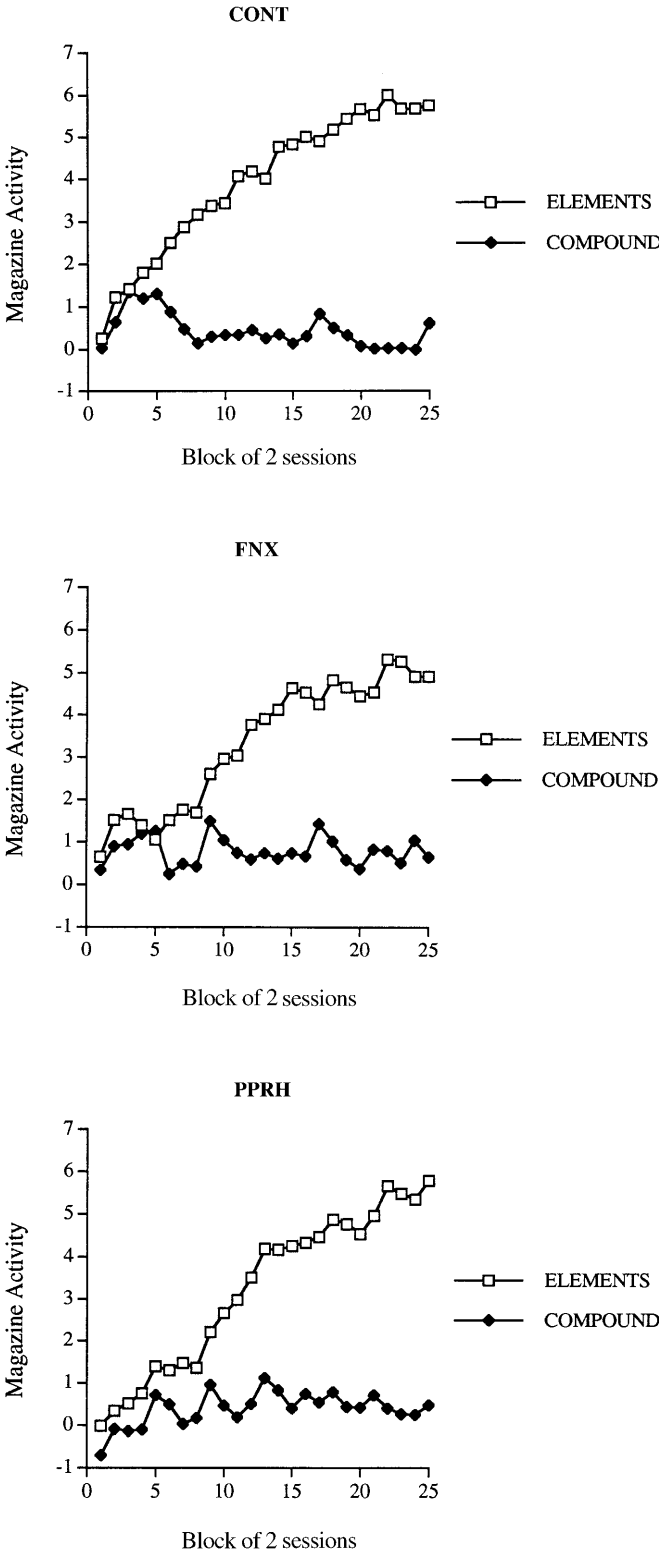

Fig. 3 Acquisition curves for the control (*CONT*), fornix-lesioned (*FNX*) and perirhinal/postrhinal cortex-lesioned (*PPRH*) rats on the negative patterning task. *ELEMENTS* refers to the A+ (tone) and B+ (darkness) stimuli. *COMPOUNDS* refers to the non-reinforced conjoint presentation of both of these stimuli (AB–). Magazine activity refers to the time spent in the food magazine during the 10-s conditioned stimulus (CS), minus the time spent in the magazine during the 10 s just preceding the onset of the CS. There were no significant differences between any of the three groups

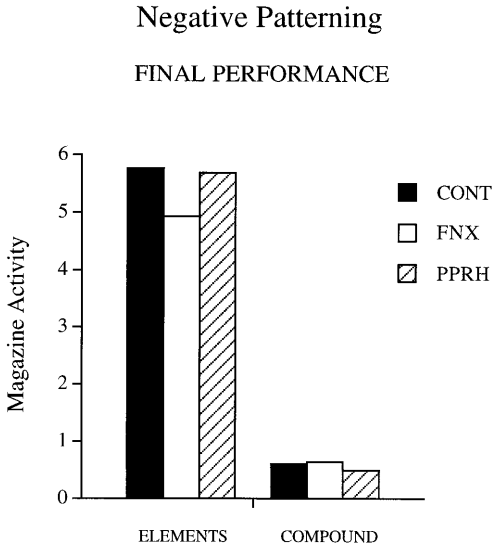

Fig. 4 Final performance levels of the *CONT*, *FNX* and *PPRH* groups on the negative patterning task. Data are from the final block of two 24-trial sessions

$F(2,12)=1.30$, $P=0.31$, and no Group×Trial interaction, $F(10,60)=1.13$, $P=0.35$. However, there was a significant main effect of Trial, $F(5,60)=13.4$, $P<0.0001$, and subsequent analysis of simple interaction effects revealed an effect of Trial for all three groups (all P values <0.01), indicating significant extinction of responding across the six extinction trials for all three groups.

Autoshaping

Subsequent testing protocols and apparatus were not able to accommodate the number of rats used in the negative patterning tests. For this reason a smaller cohort of 27 animals was retained for use in subsequent tests (CONT $n=9$, FNX $n=6$ and PPRH $n=12$), and the remaining 3 animals were used for preliminary histological assessment. It was ensured that the performance of the retained animals was very similar to that of those excluded. The FNX rat that had required further magazine training was added back into the FNX group at this point. The group sizes for this phase of the study onwards were thus CONT $n=9$, FNX $n=7$ and PPRH $n=12$.

Discriminated approach. Difference scores (approaches to the CS+ minus approaches to the CS–) were analysed across the 20 Blocks of five stimulus presentations. There was no significant effect of Group, $F(2,25)=1.83$, $P=0.18$, and no Group×Block interaction, $F(38,475)=1.20$, $P=0.20$. There was, however, a significant main effect of Block, $F(19,475)=8.48$, $P<0.0001$, and analysis of simple interaction effects revealed that the effect of Block was significant for all three groups (all P values <0.01). Thus animals of the CON, FNX and PPRH groups were able to acquire discriminative approach in this paradigm, and there were no differences in their ability to do so.

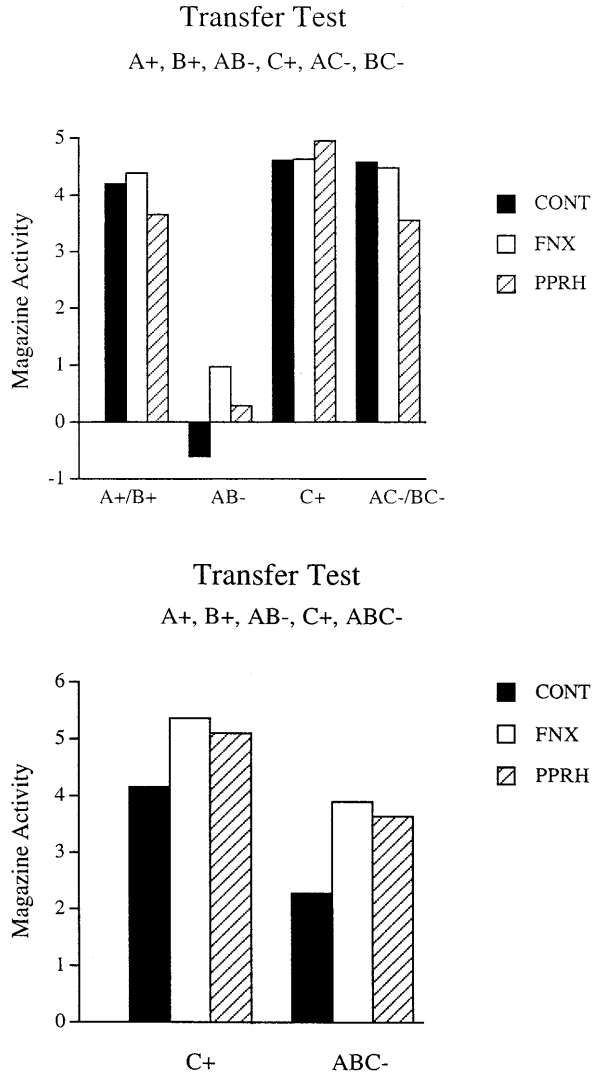

Fig. 5 Performance of the *CONT*, *FNX* and *PPRH* groups during transfer tests administered following acquisition of the negative patterning task. Magazine activity refers to the time spent in the food magazine during the 10-s CS, minus the time spent in the magazine during the 10 s just preceding the onset of the CS. *Top panel* The lack of suppression of responding to the compounds AC– and BC– provides evidence that rats did not acquire the negative patterning problem using non-configural cues such as intensity or numerosity. There were no significant differences between any of the three groups. *Bottom panel* To ensure validity of the transfer test in A, it was important to show that rats were able to suppress responding in the presence of compounds involving the clicker. That responding to C+ was greater than to ABC– provides such evidence. There were no significant differences between any of the three groups

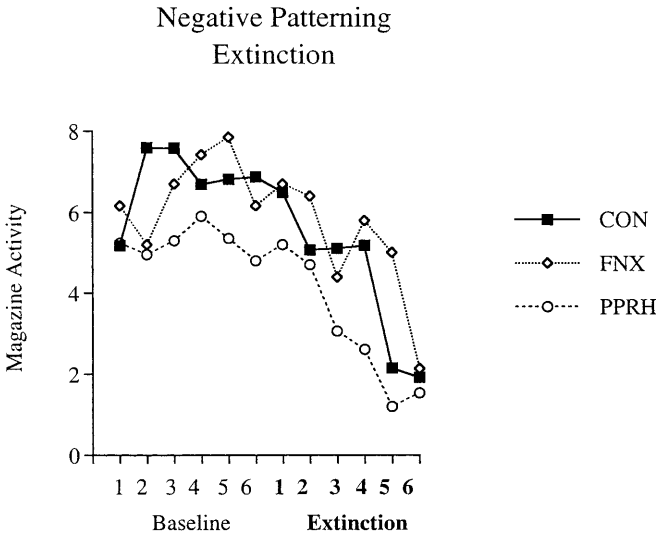

Fig. 6 Performance of the *CONT*, *FNX* and *PPRH* groups during an extinction test administered following acquisition of the negative patterning task. The *first six points* represent data from baseline trials in which A and B were reinforced. The *last six points* represent data from extinction trials in which A and B were not reinforced. Magazine activity refers to the time spent in the food magazine during the 10-s CS, minus the time spent in the magazine during the 10 s just preceding the onset of the CS. There were no significant differences between any of the three groups

Discriminated magazine approach. Similarly, there were no differences between groups in discriminated magazine approach, $F(2,25)=0.04$, $P=0.96$. There was, however, a significant main effect of Block, $F(38,475)=2.20$, $P<0.01$. There was no significant Group×Block interaction, $F(38,475)=1.39$, $P=0.07$. However, analysis of simple interaction effects revealed that while the effect of Block was significant for the CONT and PPRH groups ($P<0.01$ and $P<0.05$, respectively), for the FNX group there was no significant effect of Block ($P=0.24$).

Approach latencies. Because animals often made no approaches to the CS– within 10 presentations, we calculated the average approach latencies by taking the mean of the 100 presentations of the CS+ and CS–. There was a significant main effect of Group, $F(2,25)=3.67$, $P<0.05$, and Newman-Keuls *post hoc* comparison revealed that this was due to animals of the FNX group responding faster overall than did control animals ($P<0.05$). There was no significant Group×Stimulus interaction, $F(2,25)=0.98$, $P=0.91$, and analysis of simple interaction effects revealed a significant effect of Stimulus for each of the CONT, FNX and PPRH groups (all P values <0.01). Thus, all groups demonstrated discrimination of the CS+ and CS– according to the latency measure, responding more rapidly to the CS+ than to the CS–.

Experiment 2: object and spatial memory

Introduction

In experiment 1 it was shown that neither fornix nor combined perirhinal/postrhinal cortex lesions affected acquisition or performance of negative patterning, subsequent transfer tests, responding in extinction or stimulus-reward learning in a Pavlovian autoshaping paradigm. The present experiment tests the functional efficacy of

the lesions by testing the same rats on tests of object recognition and spatial memory that have been shown previously to be impaired by these same lesions (Aggleton et al. 1997; Bussey et al. 1999, 2000).

Materials and methods

Subjects

Subjects were the same as those in experiment 1. Animals were provided with free access to water and by means of a restricted feeding regimen were maintained at or above 85% of their free feeding weight during testing on the spatial tasks. During object recognition and locomotor activity tests, food was given ad libitum.

T-maze alternation

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

Procedure. Each animal was given a minimum of four, 5-min pretraining sessions. By the end of these sessions the rats would run down the stem of the maze to find food pellets in the food wells in both arms. Following this, the experiment proper began. At the start of each trial, which consisted of two stages, three food pellets (45 mg) were placed in each food well and a metal barrier was placed at the neck of the T-maze so closing off one arm. As a consequence, the animal was forced to enter a preselected arm on each "sample run" and then allowed to eat the food there. The animal was then picked up and confined in the start box for a delay of 15 s, during which the metal barrier was removed. The door to the start box was then opened and the animal allowed a free choice between the two arms of the T-maze. On this "choice run" the criteria for selecting an arm consisted of the rat placing a back foot in one of the arms. No retracing was permitted. If the rat had alternated, i.e. had entered the arm not previously visited on the sample run, it was allowed to eat the food reward before being returned to its cage. If the other arm was chosen, i.e. the same arm as visited on the sample run, the rat was confined to that arm for approximately 10 s, and then returned to its cage. The rats were tested in groups of three or four with each rat having one trial in turn, so that the intertrial interval was about 4 min. The animals received six trials a day, for a total of six sessions.

Mixed delays. Immediately following the acquisition session the rats received a further six sessions, each of six trials. For half of the trials within each session there was a delay of 15 s between the choice run and the test run, and for the other half there was a delay of 60 s. The delay on a given trial was determined pseudorandomly. During the delay period the animal remained in the start box at the base of the stem. The rest of the procedure remained unchanged.

Spontaneous recognition tests

Apparatus. The apparatus consisted of an open arena (100×100×46 cm) made of wood, the inside of which was painted grey. The floor was covered with sawdust. The arena was situated in a room containing features such as a door, light fixtures and a video camera, and a large dark screen which served to conceal the experimenter. Triplicate copies were obtained of the objects to be discriminated, which were made either of glass, plastic or metal. For any given test the pairs of objects to be discriminated were typically composed of the same material so that they could not readily be distinguished by olfactory cues. The height of the objects ranged from 6.3 to 22.0 cm, while their weight ensured that they could not be displaced by the rats. As far as could be ascertained the objects had no natural significance for the rats and they had never been associated with a reinforcer.

General procedure. All rats were given a minimum of four habituation sessions prior to the first test. For each of these habituation sessions the rat was placed in the empty arena for 10 min. Forty-eight hours later, testing began. Rats were given a series of test sessions, with a minimum interval of 48 h between each session. During these tests the experimenter did not know the group membership of the animals. The time spent exploring objects during the various tests was assessed from video recordings of the sample and test phases.

Object recognition test. Each test session consisted of two phases. In the initial sample phase, two identical objects (A1 and A2) were placed in the far corners of the arena, each 10 cm from the side wall. A rat was then placed in the middle of the arena and the total time spent exploring the two objects was determined from videotaped recordings. Exploration of an object was defined as directing the nose to the object at a distance of less than 2 cm and/or touching it with the nose. Turning around or sitting on the object was not considered as exploratory behaviour. This "sample phase" ended when the rat had explored the two identical objects for a total of 25 s.

After a delay of 15 min the rat was reintroduced to the arena ("choice phase"). The box now contained a third identical copy of the familiar object (A3) and a new object (B). These were placed in the same locations as the sample stimuli. The location of the two choice objects was counterbalanced between rats and across sessions. Thus on test 1, object A was the sample and object B was the novel alternative. For test 2 (48 h later) their roles were reversed, i.e. object B was the sample and object A was the "novel" alternative. This 48-h interval was thought to be sufficient as previous studies have shown that under these conditions rats fail to treat the sample stimulus as familiar if the delay between sample exposure and test is longer than 24 h (Ennaceur and Delacour 1988). All rats were tested in this manner with three different object sets, followed by repeated testing using the first object set, for a total of four object sets (1, 2, 3 and 1R). Following these four pairs of tests, a fifth pair of tests using a new set of objects was given as above, with the exception that a 1-h rather a 15-min delay was interposed between sample and choice.

Object location test. Unlike the object recognition test, rats were not tested on the ability to identify or distinguish between objects or to make judgements based on the familiarity of objects. Instead, normal discrimination involved rats recognising that an object had changed location. The use of information about the specific identity of the object was thus not required. Two copies of the same object (A) were used in each test. In the sample phase the rat was exposed to objects A1 and A2 which were placed in the two far corners of the arena. The sample phase was terminated when the animal had explored the objects for a total of 25 s. After a delay of 15 min the test phase began. In the test phase object A3 was placed in the same position as A1 while object A4 was placed in the corner adjacent to the original position of A2, so that the two objects A3 and A4 were now in diagonal corners. Thus both objects in the test phase were equally familiar but one had changed location. All animals received two test sessions separated by 48 h. This ensured that the position of the moved object in the test phase could be counterbalanced for each rat. The objects (A1–4) were identical unopened drink cans that had not been used in any previous test. Testing began 1 week after completion of the object recognition test.

Spontaneous locomotor activity

After completion of the above tests all rats were placed in novel test cages (56×39×19 cm) in a novel room. Activity was measured using pairs of photobeams situated 20 cm apart and 18 cm from the end of the cage (Paul Fray, Cambridge, UK). The total number of beam breaks was recorded. Data were gathered in 12 intervals of 10 min each.

Results

T-maze alternation

Single delay condition. Animals of the FNX group were severely impaired on spatial alternation in the T-maze at a delay of 15 s. Analysis of variance performed on the mean percent correct scores across the 6 days of training revealed a significant main effect of Group, $F(2,25)=74.7$, $P<0.0001$, with animals of the FNX group performing at no better than chance level. Newman-Keuls *post hoc* analysis revealed that animals of the FNX group were significantly impaired relative to animals of both the PPRH and CONT groups (both P value <0.01). PPRH and CONT groups did not differ. (Means: CONT, 81.5; FNX, 46.8; PPRH, 81.3.)

Mixed delay condition. Animals of the FNX group were severely impaired in T-maze alternation in the mixed delay condition. In contrast, animals of the PPRH group performed significantly *better* than control animals under these conditions. Analysis of variance with Group and Delay as factors revealed a significant main effect of Group, $F(2,25)=35.3$, $P<0.0001$, and a significant main effect of Delay, $F(1,25)=37.5$, $P<0.0001$. Newman-Keuls *post hoc* analysis revealed that performance of animals of the FNX group differed from that of both the CONT and PPRH groups (both P values <0.01). In addition, animals of the PPRH group outperformed control animals ($P<0.05$). Analysis of simple main effects revealed a significant effect of Group at both the 15-s and 60-s delay (both $P<0.001$). Subsequent analysis of variance performed separately on data from the 15-s and 60-s conditions revealed that while the lower performance of the FNX group relative to CONT and PPRH groups was significant in both conditions (all P values <0.01), the enhanced performance of the PPRH group reached significance in the 60-s condition only ($P<0.05$). (Means, 15-s delay: CONT, 84.0; FNX, 60.3; PPRH, 92.6. Means, 60-s delay: CONT, 71.0; FNX, 50.0; PPRH, 81.0.)

Spontaneous recognition tests

Object recognition test. Animals of the PPRH group were significantly impaired relative to control animals for the first set of objects tested. Analysis of variance performed on d1 scores (the difference in time spent exploring the novel and familiar objects for each of the two sets of objects) from the 1st min of choice exploration across the three object sets (1,2,3) and one repeated object set (1R) revealed a trend towards a group effect which narrowly missed significance, $F(2,25)=3.24$, $P=0.056$. Analysis of simple main effects, however, revealed a significant effect of group for object set 1, $F(2,25)=4.99$, $P=0.015$, but not for object sets 2, 3 or 1R. Newman-Keuls *post hoc* analysis for object set 1 revealed that the significant group difference was due to animals of the PPRH group performing at a significantly lower level than animals of both the CONT and FNX groups (both $P<0.05$). (Means, object set 1: CONT, 13.3; FNX, 20.5; PPRH, 2.9. Means, object set 2: CONT, 7.7; FNX, 11.9; PPRH, 6.0. Means, object set 3: CONT, 8.2; FNX, 12.4; PPRH, 7.1. Means, object set 1R: CONT, 7.4; FNX, 10.3; PPRH, 9.0.)

In order to investigate animals' discrimination performance further, and to shed potential light on the variable effects of lesions across testing, *t*-tests were carried out to compare obtained d1 scores with a score of zero (which would indicate no discrimination). This analysis revealed the potentially important result that animals of the CONT group discriminated highly significantly for object set 1 only, with discrimination performance becoming poorer across testing, and indeed failed to show significant discrimination on object set 3 [object set 1 $t(8)=9.46$, $P<0.001$; object set 2 $t(8)=3.84$, $P<0.01$; object set 3 $t(8)=2.03$, n.s.; object set 1R $t(8)=2.75$, $P<0.05$]. In sharp contrast, animals of the PPRH group discriminated *more* significantly as training progressed [object set 1 $t(11)=1.175$, n.s.; object set 2 $t(11)=1.78$, n.s.; object set 3 $t(11)=2.77$, $P<0.05$; object set 1R $t(11)=4.34$, $P<0.01$]. The significance levels of discrimination by FNX animals varied less systematically [object set 1 $t(6)=3.34$, $P<0.05$; object set 2 $t(6)=2.31$, n.s.; object set 3 $t(6)=1.23$, n.s.; object set 1R $t(6)=3.02$, $P<0.05$]. Thus it appears that the failure to observe significant effects of the perirhinal/postrhinal cortex lesion on discrimination learning for all but the first object set tested may be due to the discrimination performance of control animals becoming poorer, and the discrimination performance of the PPRH animals becoming better, as testing progressed.

Testing at the 1-h delay was carried out following testing at the 15-s delay, and consistent with the observation that lesion effects diminished as testing progressed (see above), there were no significant group effects under this condition, $F(2,25)=1.88$, $P=0.17$. (Means: CONT, 13.1; FNX, 5.43; PPRH, 7.2.)

Object location test. Unlike the spontaneous object recognition task, in which a novel object is discriminated from a familiar one, in the object location task an object which has changed location is discriminated from one which has remained static. Analysis of variance revealed no significant effect of Group overall, $F(2,25)=2.28$, $P=0.12$. However, analysis of simple main effects revealed a significant main effect of Group for object set 1, $F(2,50)=4.11$, $P=0.022$. Newman-Keuls *post hoc* analy-

sis revealed that this effect was due to animals of the PPRH group significantly outperforming animals of the FNX group ($P<0.05$). (Means, object set 1: CONT, 2.84; FNX, –3.96; PPRH, 9.88. Means, object set 2: CONT, 6.96; FNX, 5.75; PPRH, 6.23.)

Activity

Cage crosses and photocell breaks were analysed across 12 time intervals of 10 min each. Due to equipment malfunction, data from one rat in the FNX group was unavailable.

Crosses. There was a significant main effect of Group, $F(2,24)=7.02$, $P<0.01$. Newman-Keuls *post hoc* analysis revealed that animals of the FNX group made significantly more cage crosses than animals of either the CONT or PPRH groups (P values <0.01). There was also a significant Group×Interval interaction, $F(22,264)=4.91$, $P<0.0001$, indicating that although FNX animals were more active at the outset of the session, by the end this activity had decreased to control levels. There were no other group differences.

Photocell breaks. There was a significant main effect of Group, $F(2,24)=6.271$, $P<0.01$. Newman-Keuls *post hoc* analysis revealed that animals of the FNX group made significantly more photocell beam breaks than animals of either the CONT ($P<0.05$) or PPRH ($P<0.01$) groups. There was also a significant Group×Interval interaction, $F(22,264)=2.816$, $P<0.0001$, indicating that although FNX animals were more active at the outset of the session, by the end this activity had decreased to control levels. There were no other group differences.

Discussion

The results of the present study show that neither fornix lesions, nor combined excitotoxic lesions of perirhinal and postrhinal cortex, had any effect on acquisition or performance of the negative patterning task. Transfer tests following acquisition provided evidence that neither lesioned nor control animals solved this problem using non-configural cues such as intensity or numerosity. Further tests showed that lesioned animals were not resistant to extinction, nor were they impaired in simple conditioning using a Pavlovian autoshaping procedure. Subsequent tests of object and spatial memory demonstrated the functional efficacy of the lesions. Specifically, fornix lesions disrupted T-maze alternation but had no effect on spontaneous object recognition, and perirhinal/postrhinal cortex lesions selectively impaired object recognition but *facilitated* T-maze alternation performance when a 60-s delay was interposed. In addition, on an object location task in which normal animals recognise that an object has changed location, without having to *identify* the object that has moved, PPRH animals significantly outperformed FNX animals. Finally, FNX animals were significantly more active than control or PPRH animals in novel activity cages. All of these findings from tests of spatial memory, object memory and spontaneous activity are replications of previously reported dissociations and double dissociations between the effects of these two lesions (see, for example, Aggleton et al. 1997; Bussey et al. 1999). These dissociations argue against a functionally unitary temporal lobe memory system including the hippocampus and perirhinal/postrhinal cortex, and instead support distinct roles for these brain regions in spatial memory (O'Keefe and Nadel 1978) and object identification (Buckley and Gaffan 1998b; Murray and Bussey 1999; Murray et al. 1998), respectively. Unlike our previous studies (Aggleton et al. 1997; Bussey et al. 1999, 2000), however, in the present study the effect of perirhinal/postrhinal cortex lesions on the object recognition task was transient. The transient nature of this effect, discussed in more detail below, does not affect interpretation of the negative patterning results, as acquisition of the negative patterning task occurred prior to the tests of spatial and object memory.

Fornix lesions

The issue of whether hippocampus or fornix lesions can impair negative patterning has been controversial. Davidson et al. (1993) found no impairment using a procedure that controlled for response rate, suggesting that previously reported impairments may have been due to overresponding by the lesioned animals. McDonald et al. (1997), however, argued that the experiments in the study of Davidson et al. (1993) failed to control for the excitatory properties of reward. McDonald et al.'s own study, however, did not control for response rates; nor did it include transfer tests to show that animals were not using non-configural solutions to the problem. The present study focused on the effects of fornix lesions on negative patterning using a paradigm that controlled for all of these possible confounds. The results provide strong evidence that fornix lesions can completely spare negative patterning in rats.

It appears to be highly problematic for configural association theory (Rudy and Sutherland 1995; Sutherland and Rudy 1989), which views spatial learning as a special case of configural learning, that fornix lesions, which reliably disrupt spatial learning, had no effect on configural learning (see also Bussey et al. 1998). A caveat, however, is in order. One possibility that is compatible both with our results and with configural association theory is that the fornix contributes to some facet of spatial learning tasks that is less important in configural tasks. An obvious possibility is the different response requirements of these two types of task. Specifically, navigation towards a relatively distant goal, which is a requirement for all of the spatial tasks under discussion, is not a requirement in configural tasks which are usually

carried out in operant chambers. Thus a model that might be compatible both with our results and with configural association theory is one in which the hippocampus mediates configural and spatial information processing and the fornix contributes to the translation of this information into navigation to a goal, via its role in connecting the hippocampus with subcortical regions. Such regions may include the nucleus accumbens, anterior thalamus and mamillary bodies, lesions of which can disrupt acquisition of spatial tasks (see, for example, Aggleton and Brown 1999; Aggleton et al. 1996; Sutherland and Rodriguez 1989).

Indeed, McDonald et al. (1997) have suggested that hippocampal, but not fornix lesions, impair negative patterning, a result consistent with the foregoing model. Unfortunately, the possibility of ceiling effects in that study precludes concluding unequivocally that fornix and hippocampal lesions differentially affect negative patterning. This is because the hippocampal control animals, to which hippocampal lesioned animals' performance was compared, responded at higher rates than the fornix control animals in the corresponding fornix lesion experiment. Thus although both fornix and hippocampal-lesioned animals overresponded relative to their own controls, because of the high response rates of *all* animals in the hippocampal experiment, hippocampal-lesioned animals would have had to respond at over 80 lever presses per minute in order to evidence discrimination. Indeed inspection of the data reveals evidence that hippocampal-lesioned animals were beginning to discriminate, up to the point at which their levels of responding reached this putative ceiling.

Other recent results argue more directly against the idea that the hippocampus is the seat of configural learning. For example, Davidson et al. (1993) and Deacon and Rawlins (1996) reported no effect of hippocampal lesions on configural learning, and Richmond et al. (1997) found only transient effects on negative patterning. More recently, Dusek and Eichenbaum (1998) reported no effect of fornix lesions on the initial acquisition of an olfactory version of the transverse patterning task (although deficits did emerge under one condition later in training), and Bussey et al. (1998) showed that fornix lesions could *facilitate* acquisition of transverse patterning. Other hippocampal studies are somewhat difficult to interpret because impaired "elemental" learning can occur in the same paradigms as those used to observe deficits in configural learning (see, for example, Alvarado and Rudy 1995). In summary, unequivocal evidence has yet to be presented showing that the hippocampal system mediates all forms of configural learning. It also remains unclear whether there is any relationship between configural and spatial learning. Indeed, the more general issue of whether the brain is dichotomised into separate elemental and configural learning systems at all, awaits confirmatory evidence. In the absence of such evidence, we would advocate a more parsimonious model in which "configural" and "elemental" learning are anatomically coextensive.

Perirhinal/postrhinal cortex lesions

This is, to our knowledge, the first study to investigate the effects of lesions of the parahippocampal region on configural learning in rats. The results show that, at least in the case of negative patterning with simple light and tone stimuli, such lesions have little effect. McDonald et al. (1997) have suggested that configural learning is mediated by the hippocampus and "retrohippocampal" connections, without involvement of the fornix. The lack of effect of perirhinal/postrhinal cortex lesions – which in addition to the perirhinal/postrhinal cortex included lateral entorhinal cortex and much of area TE – on negative patterning in the present study appears to rule out at least one retrohippocampal route. As suggested by McDonald et al. (1997), however, it is conceivable that spared hippocampus–posterior cingulate connections could mediate this type of learning (Sutherland and Hoesig 1993). Further lesion experiments would be required to evaluate this suggestion.

That perirhinal/postrhinal cortex lesions did not disrupt negative patterning seems at odds with a recent study (Buckley and Gaffan 1998a) showing that perirhinal lesions in the monkey can disrupt configural learning. A recent neural network model, however, predicts that although visual representations are bound in perirhinal cortex into 'configural' units, these units represent complex 'objects', with representations of simple features of objects stored in cortical regions upstream from perirhinal cortex (Murray and Bussey 1999; Saksida and Bussey 1998). Thus the model predicts that configural learning will be disrupted by perirhinal cortex lesions when complex but not simple stimuli are used, a prediction in line both with the results of Buckley and Gaffan (1998a) and with those of the present study.

Another possibility is that negative patterning could be supported by the medial entorhinal cortex, which was spared in every case in the present study. Indeed, entorhinal cortex lesions have been shown to have similar effects to perirhinal cortex on some tasks (Meunier et al. 1993). Although this possibility cannot be ruled out, it seems unlikely in light of the finding that perirhinal cortex lesions that spare entorhinal cortex can disrupt configural learning (Buckley and Gaffan 1998a).

Tests of spatial and object recognition memory

It has previously been shown that lesions of the perirhinal/postrhinal cortex lead to impairments on the spontaneous object recognition task but not on tests of spatial memory, whereas fornix lesions can produce the opposite effect (Aggleton et al. 1997; Bussey et al. 1999, 2000). The present study replicated this pattern of results. Whilst the data from the first object set clearly indicate differential effects of fornix and perirhinal/postrhinal cortex lesions, however, the lack of impairment on subsequent object pairs is puzzling. One possibility is that the effects of perirhinal/postrhinal cortex lesions depend critically on

the nature of the objects used. It is possible that certain object pairs may be discriminated according to simple features such as size, and therefore do not require perirhinal/postrhinal cortex which may be specialised for the identification of complex objects (Buckley and Gaffan 1998b; Murray and Bussey 1999; Murray et al. 1998; Saksida and Bussey 1998). Perhaps more likely is that the failure to observe perirhinal/postrhinal cortex-lesion effects on later object sets was due to floor effects in the control group, which discriminated the novel objects from the familiar objects highly significantly for the first object set only (and in one case failed to discriminate). Alternatively, the lack of effect of the perirhinal/postrhinal cortex lesion on later object sets could be related to recovery of function (Ennaceur and Aggleton 1997). Importantly, the possibility of recovery of function does not affect interpretation of the negative patterning results, as the acquisition of the negative patterning task occurred prior to the tests of object recognition.

Conclusions

The present study tested in a controlled manner the hypothesis that lesions of the fornix, and combined lesions of the perirhinal/postrhinal cortex, would impair configural learning. Clear contradictory evidence was found. At the same time these lesions had a detrimental effect on spatial memory and object recognition, respectively. These results add to a growing body of evidence that casts doubt on the popular notion that spatial learning is a special case of configural learning, and that the hippocampus and related structures are necessary for this type of learning. Future studies will need to examine what brain regions are necessary for configural learning, and if they differ from those comprising a putative "elemental" learning system.

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