Fornix–Fimbria Section and Working Memory Deficits in Rats: Stimulus Complexity and Stimulus Size

Helen J. Cassaday and J. N. P. Rawlins
University of Oxford

Rats were trained on delayed matching-to-sample (DMS) with goalboxes containing complex objects as stimuli. On reaching the preoperative learning criterion, the rats were allocated to conventional fornix-lesioned or control groups. In a series of postoperative DMS experiments, different kinds of stimuli were used, ranging from complex object boxes to large, simple black or white goalboxes, with 3 transitional types in between. Lesions impaired choice accuracy whenever the rats were tested with large, simple goalboxes, but not with smaller boxes of otherwise identical construction. A brief, final experiment showed no amelioration of the lesion-induced impairment when complex objects were added to large, simple goalboxes. The results are discussed in terms of spatial and nonspatial accounts of hippocampal function.

There is widespread agreement that the hippocampal formation plays a critical role in normal memory processing, but considerable disagreement on the best theoretical account of that role. One clear distinction in principle is between theorists advocating an exclusively spatial role for the hippocampal formation in learning and memory (Nadel, 1991; O'Keefe & Nadel, 1978; Worden, 1992) and those preferring a nonspatial account (Olton, Becker, & Handelmann, 1979; Rawlins, 1985; Sutherland & Rudy, 1989).

There are several demonstrations that conventional lesions of the hippocampus or fornix–fimbria can impair performance on nonspatial matching or nonmatching tasks (Jagiello, Nonneman, Isaac, & Jackson-Smith, 1990; Olton & Feustle, 1982; Raffaele & Olton, 1988). These might be expected to have ruled out any exclusively spatial theory of hippocampal function. One reason why that has not happened arises from the data obtained from other studies using comparable lesions; these data show performance impairments on a variety of spatial memory tasks, coupled with spared performance on task variants that were intended to be analogous, but that depended on using nonspatial instead of spatial cues (Aggleton, Hunt, & Rawlins, 1986; Morris, Garrud, Rawlins, & O'Keefe, 1982; Parkinson, Murray, & Mishkin, 1988). These and related findings (Mumby, Wood, & Pinel, 1992) might be thought to suggest that nonspatial deficits resulting from conventional hippocampal damage are at best capricious, and therefore pose little threat to exclusively spatial theories of hippocampal function.

This suggestion is weakened by recent findings from a series of within-subjects experiments that assessed nonspatial working memory performance in rats with fornix sections or hippocampal aspiration lesions (Rawlins, Lyford, Seferiades, Deacon, & Cassaday, 1993; Yee & Rawlins, 1994). The first of these studies used a discrete-trial delayed matching-to-sample (DMS) design, using goalboxes containing complex objects (Aggleton, 1985), or plainer goalboxes that differed mainly in their surfaces' paint finish and texture. The results showed the presence of a lesion-induced impairment only when the stimuli to be remembered were used repeatedly within sessions rather than in a pseudo-trial-unique design, and that the impairment was greatest when the repeatedly used stimuli were the plain ones. Yee and Rawlins (1994) used a delayed nonmatching-to-sample (DNMS) design and showed an impairment when plain goalboxes were used in a design in which repetition was not a factor because there was only one trial per day. Once these factors are taken into account, the results of the nonspatial DMS and DNMS experiments reviewed above fall neatly into place.

The overall pattern of the data is consistent with the possibility that hippocampal dysfunction leads to increased sensitivity to within-trial and between-trial interference but leaves performance essentially unaffected when complex objects are used as stimuli in a trial-unique design. This latter sparing of performance is itself consistent with recent suggestions that deficits in visually guided object-matching tasks in monkeys, which were formerly attributed to hippocampal dysfunction, should rather be attributed to rhinal area dysfunction (Gaffan & Murray, 1992; Murray, 1992; Zola-Morgan, Squire, Amaral, & Suzuki, 1989). If rhinal cortex function is equivalent in rats and in monkeys, then when object matching in a (pseudo-) trial-unique design is unaffected, the rhinal area may be presumed to be functioning normally; if at the same time DMS or DNMS performance is impaired when plainer goalboxes are used instead of complex objects, then this impairment presumably arises from dysfunction outside the rhinal area. Thus it seems plausible that damage to the hippocampus or its connections underlies the results reviewed above.


This research was supported by the Wellcome Trust (Project Grant 036159), with additional support from the MRC Centre for Brain and Behaviour and the McDonnell-Pew Foundation for Cognitive Neuroscience. We would like to thank R. M. J. Deacon and A. Kacelnik for helpful discussion.

Correspondence concerning this article should be addressed to Helen J. Cassaday, University of Oxford, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD, England. Electronic mail may be sent via Internet to cassaday@vax.ox.ac.uk.

594
However, there is a further possible discrepancy with the primate literature. Monkeys with conventional hippocampal lesions have been shown to perform normally on a DMS that only used two small, colored disks as stimuli (Correll & Scoville, 1965). This appears inconsistent with Rawlins et al.'s (1993) observation that rats with lesions of this kind perform poorly on DMS using two simple goalboxes as stimuli. They suggested that this discrepancy may also be resolved if the nature of the simple stimuli actually used is taken into account (small, discrete stimuli in the primate experiments; larger and more diffuse stimuli in the rodent experiments; for discussion see Rawlins et al., 1993). They concluded that according to this view, rats with conventional hippocampal system lesions should more successfully perform a DMS when tested with otherwise identical complex objects that differed only in being painted black or white than when tested with large, plain goalboxes that also differed only in being painted black or white (Rawlins et al., 1993, pp. 431-432).

In the present series of experiments, we therefore used a DMS that was essentially identical to the one used earlier (Rawlins et al., 1993), except that the nonspatial stimuli used here ranged from complex objects to large, plain, black or white goalboxes, with transitional forms in between. We assessed the performance of rats with fornix-fimbria sections because we have consistently found that hippocampectomy and forniciotomy have identical effects on these kinds of tasks (Rawlins et al., 1993; Yee & Rawlins, 1994), but hippocampal lesions entail more ancillary damage. We anticipated a graded increase in the impairment induced by fornix section as the stimuli to be matched were systematically varied from complex objects at one extreme to large, plain goalboxes at the other. Initially, two transitional forms of stimuli were used. These were modifications of the goalboxes used for pseudo-trial-unique testing with complex objects: (a) a pair of goalboxes containing identical objects, differing only in being painted entirely black or entirely white, and (b) a pair of empty goalboxes of the same kind, painted entirely black or entirely white. The point in this sequence at which performance was disrupted would identify which features of the complex object stimuli (Aggleton, 1985; Aggleton et al., 1986; Rawlins et al., 1993) are critical for successful DMS performance in lesioned animals under testing conditions resembling those used by Raffaele and Olton (1988). In later experiments in the series further modifications of goalbox design were employed.

The rats were trained preoperatively in a discrete-trial DMS procedure using a single pair of complex objects as stimuli, because the effects of stimulus complexity could only be assessed using repeated stimulus presentations. Postoperatively they were tested with pairs of stimuli of the four levels of complexity described above. These four conditions were run on a 5-day rotation of stimulus type, with the 5th day being a pseudo-trial-unique condition that was predicted to be easiest for all groups because of the reduced potential for interference (see Rawlins et al., 1993).

The experiments aimed first to identify those critical stimulus features that make nonspatial working memory tasks insoluble for rats with fornix sections and second to address explicitly the possibility that these lesions increase sensitivity to within-trials interference. The results should therefore address both the specific issue of comparability between rodent and primate studies and the more general issue of how best to describe the psychological consequences of conventional hippocampal system lesions. The further question of how to identify the critical neural substrate whose damage underlies these effects would require the assessment of rats with selective cytotoxic lesions; the present study does not address that issue.

**General Method**

**Subjects**

The subjects were 30 experimentally naive male Dark Agouti rats obtained from Bantin and Kingman (Hull, England), weighing 220-245 g at the beginning of preoperative training and 215-245 g at surgery. They were housed in a temperature-controlled room (23°C), on a 12-hr light–dark cycle (lights on at 7 a.m.), caged in pairs preoperatively but alone postoperatively to allow better weight control. They each received 10-20 g of rat chow (Witham, Essex, UK) upon the completion of the day's testing, except for a 2-week postoperative recovery period during which they were maintained on full food. Thereafter the amount of food was individually adjusted for each rat, to keep their weights to at least 80% (range 81%-96%) of their preoperative value throughout testing. Access to water was unlimited throughout.

One rat died during preoperative training: All the rats were periodically treated with the antibiotic Clamoxyl in order to manage a recurrent chest infection over the course of the experiment. Five rats did not meet the preoperative training criterion and were dropped from the experiment. All experiments were run during the light phase.

**Surgery**

A total of 24 rats, matched for preoperative performance scores, were allocated to one of three surgical groups, fornix sectioned, sham lesioned, and sham operated. The rats were anesthetized by intraperitoneal injection of Avertin at a dose 10 ml/kg (Avertin concentrate consists of 100 g of 2,2,2-tri-bromo-ethanol dissolved in 62 ml tertiary amyl alcohol; 1.25 ml of this concentrate are added to 5 ml absolute alcohol and 62.5 ml of 0.9% saline). Seven rats died under anesthesia as a consequence of respiratory problems presumably due to underlying chest infection. Surgery was performed on a Kopf stereotactic instrument (Tujunga, CA), with skull flat between bregma and lambda. The right temporal muscle was retracted and a hole drilled through the side of the skull. For the fornix lesion (n = 9), a fine watchmaker's forceps, specially ground and held horizontally on the stereotactic manipulator with its tips 1.5 mm apart, was inserted at 1 mm posterior to bregma, with its lower tip 5.3-5.5 mm deep from bregma; it was moved using the screw drive to a point in the contralateral hemisphere 4 mm past the midline, as defined by the position of bregma. The following adjustments to depth were made for weight: 200-240 g (n = 5), 5.3 mm; 240-260 g (n = 12), 5.4 mm; 260-300 g (n = 7), 5.5 mm. To section the fimbria, the forceps was clamped with a screw, held shut for 2 min, and then opened and retracted. There were two control conditions: The sham-lesion group (n = 4), underwent the same surgical procedure, except that the forceps was only inserted 4 mm from the dura and was not closed; the sham-operated group simply had the skull exposed under anesthetic, as above. For the lesioned and sham-lesioned groups, sterile gelatin foam soaked in sterile physiological saline was laid over the exposed dura; for all three groups the wound was sprinkled with sulphonamide powder before the scalp was sutured, and 40,000 U of Bicillin (Broacdes, West Byfleet, Surrey, UK) was injected into the femoral muscle.
Histology

At the end of Experiment 6 all of the rats were given an overdose of sodium pentobarbitone, and perfused through the heart with 0.9% saline, followed by 10% formal saline. The brains were removed and stored in 30% sucrose-formalin for several weeks. They were then embedded in Cryo-M-Bed (BDH, Merck, Lutterworth, Leicestershire, UK), frozen in isopentane over solid CO₂ and cut into 50-μm coronal sections on a cryostat. Every second section was saved and stained with Weil’s Iron Haematoxylin. The sections were examined microscopically in order to assess the lesions. All the brains were histologically assessed, including the unoperated control rats.

Apparatus

The apparatus (Rawlins et al., 1993) was constructed of sheet aluminum alloy and was modified after that used by Raffaele and Olton (1988). It comprised a start area which expanded into two goal areas. The walls of the apparatus were 20.3 cm high. The start area and each of the goal areas were covered by hinged Plexiglas lids; there was a hole above each goal area through which reward pellets (two 45-mg Noyes reward pellets; Lancaster, NH) could be dropped. The start area measured 13.5 cm wide and 27.9 cm long. The end of this space, which opened into the goal areas, could be closed with a guillotine door. The two goal areas, each 13.9 cm wide and 33.2 cm long, lay side by side, being separated by a common wall. They were open at both ends. The end facing the start area could be closed with a guillotine door. The other open end allowed for the insertion of the removable goal boxes. Each goal area was illuminated by three 2.8-W incandescent bulbs mounted on the outside of the Plexiglas lids. The apparatus rested on a table in a brightly lit room.

Stimuli

The stimuli used in Experiments 1-4 ranged from goalboxes containing complex objects, to two transitional forms, to large, plain goalboxes, and are described below in that order. Additional stimuli were used in Experiments 5 and 6, and are described there. Box-type abbreviations and conditions of presentation in the different experiments are also summarized in Table 1.

Complex object boxes (comp-objects). A set of 52 goalboxes were modeled after those used by Aggleton (1985). Each of these complex stimuli differed in texture, colored paint design, and the objects they contained. The goalboxes were constructed of hardboard, with a base 17.3 cm long, side walls 8.5 cm long and 15.8 cm high, and a back wall 11.7 cm wide and 15.8 cm high. A top covered the area enclosed by the side walls. A single pair of these goalboxes was used throughout preoperative training and postoperative testing in Experiment 1. A set of 40 comp-objects was used in Experiments 2 and 3, and another pair was reserved for use in Experiment 4.

Black or white object boxes (b/w-objects). These were a pair of comp-object-sized boxes containing identical objects in identical configurations. They differed only in paint finish: One was entirely matte white and the other was entirely black. The b/w-objects were introduced in Experiment 2 (Phase B) and used in Experiment 3.

Black or white shells of complex boxes (b/w-shells). These were a pair of box shells of the same dimensions as the two forms of object boxes described above, but with no objects in them. One was matte white and the other was black. Shells were introduced in Experiment 2 (Phase B) and used in Experiment 3.

Black or white simple goalboxes (long-simple). Four simple goalboxes were modeled after those of Raffaele and Olton (1988); for an illustration see Rawlins et al. (1993). They were constructed of plywood and were longer than the goalboxes described above. Their dimensions were 25.4 cm long and 12.0 cm wide, with side walls 12.0 cm high and the rear wall 16.0 cm high (the end towards the start area was open). Long-simples differed only in paint finish, one being matte white and the other black in each pair. The first pair was introduced in Experiment 2 (Phase B), used throughout Experiment 3, and later modified for use in Experiment 6. The second pair of long-simples was used in Experiment 4.

Preoperative Training

Phase 1: Shaping. The animals were handled daily for 2 weeks and introduced to reward pellets in the home cage over 2 days as food deprivation was introduced. During the 3rd week, the animals were placed in pairs (cagemates) in the two-choice apparatus (ends blocked off with blank goalboxes) and were encouraged to explore by the placement of reward pellets throughout the maze. On successive days, the number of pellets was decreased and their location was gradually restricted to the goalboxes. The metal guillotine doors were occasionally lowered so that the rats would habituate to the noise and feeling of the door on their tail.

A total of six introductory trials was given over the 2 days preceding the start of the experiment proper, so that rats reluctant to run could be identified and given additional exposure to reward pellets on the maze. Eight of the 30 rats required extra exposure to the maze before they would run freely under experimental conditions (detailed in Phase 2, below).

Phase 2: Repeated comp-objects. After the rats were familiarized with the apparatus, they were trained on the DMS rule. A single pair of comp-object boxes was used in a discrete trial procedure throughout acquisition. The pair of boxes chosen had been used in previous studies and had been judged matched for unconditioned preferences.

For testing, 6 rats were removed from their home cages and were placed in separate holding cages in the testing room. Each rat received

<table>
<thead>
<tr>
<th>Box-type abbreviation</th>
<th>Presentation condition</th>
<th>Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Comp-objects</td>
<td>PTU</td>
<td>2, 3</td>
</tr>
<tr>
<td>(Complex object boxes)</td>
<td>Repeated</td>
<td>1, 3, 4</td>
</tr>
<tr>
<td>B/W-objects</td>
<td>PTU</td>
<td>2 (Phase B)</td>
</tr>
<tr>
<td>(Black or white object boxes)</td>
<td>Repeated</td>
<td>3</td>
</tr>
<tr>
<td>B/W-shells</td>
<td>PTU</td>
<td>2 (Phase B)</td>
</tr>
<tr>
<td>(Black or white shells of complex boxes)</td>
<td>Repeated</td>
<td>3</td>
</tr>
<tr>
<td>Long-simples</td>
<td>PTU</td>
<td>2 (Phase B)</td>
</tr>
<tr>
<td>(Black or white simple boxes)</td>
<td>Repeated</td>
<td>3, 4, 5</td>
</tr>
<tr>
<td>Short-simples</td>
<td>Repeated</td>
<td>5</td>
</tr>
<tr>
<td>(Shortened black or white simple boxes)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Object-simples</td>
<td>Repeated</td>
<td>6</td>
</tr>
<tr>
<td>(Black or white simple boxes containing objects)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note. PTU = pseudo-trial-unique stimulus presentations, that is, no goal box presented more than once on any one day. Repeated = no more than two goalboxes presented on any one day. Experiment 2 (Phase B) was used to introduce new boxes (later used repeatedly); Novel boxes were inserted in the PTU sequence with comp-objects.
only one trial at a time; thus, the 1st rat would receive its second trial only after the other 6 rats had received their 1st (discrete trial procedure). There were five groups of 6 rats.

A trial consisted of a forced (information) run and a choice (recognition) run. The rat was placed in the start area for 5 s, after which the door was slowly raised over 1 s and the rat could enter one of the goal areas (the door to the other being closed). Once the rat had entered, the guillotine door to that area was lowered. The rat was allowed to explore the goalbox until it found at least one of the two reward pellets that had been dropped through the hole above the goalbox area onto the goalbox base (occasionally pellets were inaccessi-
ble). The typical goalbox confinement was 5 to 7 s to find both pellets. The rat was then returned by hand to the start area. The intertrial interval (time between completion of forced run and beginning of choice run) was 3 to 5 s. This delay reflects the time required to move the rat from one compartment of the maze to another and to alter box positions if necessary or to remove and replace the boxes when the box positions were to be held constant. The intertrial interval was an estimated average of 6 to 7 min.

On the choice run, all doors were raised and the rat was allowed to choose between the two goalboxes. The choice criterion was that the rat should place at least three paws simultaneously on the goalbox. When this criterion was met, the door to that goal area was lowered. If the rat returned to the box entered on the forced run (match-to-
sample), it was rewarded with two pellets, delivered as above. The rat was returned to its holding cage, and the next rat began its trial. If the incorrect box was selected, from Day 5 onwards a within-trial correction procedure was used to help to avoid the development of side preferences: This applied to the first four trials each day. The door was not closed after an incorrect choice and the rat was allowed to enter the other arm and obtain rewards. On the later trials each day, an incorrect choice led to nonrewarded confinement in the goalbox for 5 to 7 s.

The design was fully counterbalanced throughout. The left–right positions of forced runs and of correct choice runs were switched pseudo-randomly across trials. The left–right goalbox positions were altered between the forced and choice runs on half of the trials (also ordered pseudorandomly). Thus the rat learned a cue-relevant/space-
irrelevant task. The comp-object stimuli were designated correct (S+) or incorrect (S-) in a balanced semirandom sequence, with the constraint that the same box was not S+/S- for more than two consecutive runs.

The animals were tested in the same order, 5 days per week, at approximately the same time every day between 9 a.m. and 5 p.m. Each rat received the same sequence of left–right goalbox positioning. Eight trials per rat were given on each of the next 68 days.

The mean scores from the last two blocks of 4 days were used to balance performance levels between groups before surgery. Five rats were excluded at this stage for poor performance. The mean choice accuracy over the last two blocks of preoperative training ranged from 80% to 99% for the remaining 24 rats.

Postoperative Testing

Seventeen rats were tested postoperatively. All testing was conducted with the experimenter uninformed as to the rats' allocation to the experimental groups.

The rats were handled during the postoperative recovery period to reduce general reactivity and then given an additional day of apparatus exposure, as described in the Preoperative Training section. Rats were reacustomed to the maze, to the noise of the lid and doors, and to confinement in the holding cages. They were then retrained on the DMS rule. Procedures throughout the study were identical to those in Phase 2 of the Preoperative Training section, except for experimental manipulation of the nature of the stimuli and their repetition (re-
peated compared with pseudo-trial-unique stimulus presentations), and the number of trials per day was increased from 8 to 12 after Experiment 1.

Statistics

Analyses were carried out using the GENSTAT IV package imple-
mented on a VAX computer. The dependent variable was percentage correct. To analyze Experiments 1-6, we used a nested design in which forces penetration (Surgery) was nested within the major factor of Lesion (with two levels, fornix-sectioned and control). Because there were no significant interactions involving Lesion × Surgery, sham-operated and sham-lesioned control groups were combined into a single control group against which to assess the effects of fornix section. Significant interactions were explored with post hoc compari-
sions made by t test, using the standard error of the difference of the mean derived from the corresponding stratum of the analysis of variance (ANOVA).

Experiment 1

This experiment assessed the effects of fornix section on retention of the preoperatively acquired DMS, using the same single pair of comp-object stimuli throughout each test session.

Method

There was one block of 4 days training with the same pair of comp-object boxes used in preoperative training. Rats were given eight trials per day in three groups of 5 to 6 rats. Procedures were identical to those described for preoperative training.

Results

Histological. Fornix-fimbria lesions were made using surgical procedures identical to those that have been described elsewhere (e.g. Feldon, Rawlins, & Gray, 1985; Rawlins et al., 1993; Yee & Rawlins, 1994). Microscopic examination re-
vealed very substantial damage to the fornix-fimbria in all rats in the lesion group, with very limited sparing in two rats only. There was no direct damage to the corpus callosum or thalamus, although the corpus callosum appeared stretched and thinned in several rats as a result of ventricular enlarge-
ment (see Figure 1). The insertion and closure of the forceps caused restricted ipsilateral neocortical damage, and clear ipsilateral damage to the caudate putamen in the fornix-
sectioned group. Insertion alone caused minimal damage to neocortex in the sham-operated group. There was no damage to the rostral pole of the hippocampal formation, but there was direct damage to the septal nuclei in 3 rats; in 2, damage was restricted to the posterior septum. The ventricles were en-
larged not only in the fornix-sectioned and sham-operated control groups, but also in the unoperated controls: This suggests that some tissue shrinkage occurred during histologi-
cal preparation, exaggerating any ventricular enlargement that may have resulted from surgery. No rats were excluded on histological grounds. A coronal section from 1 rat from each experimental group is shown in Figure 1.

Behavioral. Mean percentage correct was 84.2% over the 4 days of retraining with repeated comp-object boxes. Using an ANOVA, we compared performance on the final block of 4
days of preoperative acquisition to performance on the first block of 4 postoperative days, which used identical methods and stimuli. The analysis confirmed that postoperative performance was not significantly different from that on the final block of preoperative training (87.3%). There was no significant effect of Lesion, $F(1, 14) = 1.95$, or of Blocks, $F(1, 14) = 1.04$, and no significant Lesion × Blocks interaction, $F(1, 14) = 1.74$.

Discussion

We have previously reported that fornix-sectioned rats show an acquisition deficit when trained postoperatively using repeated comp-object stimuli (Rawlins et al., 1993). In the present experiment no lesion-induced deficit was observed. The present study differed from our earlier one in that there was substantial preoperative training, and correction procedures continued to be used during this part of postoperative testing. It seems possible that once rats have had substantial training on the DMS using repeated comp-objects as stimuli, they can still choose accurately despite having substantial fornix lesions. It has already been suggested that with postoperative training which systematically increases repetition of comp-objects within sessions, rats with fornix-fimbria lesions perform as accurately as controls on DNMS tasks (Shaw & Aggleton, 1993).

Experiment 2

We next assessed the effects of fornix section on DMS using stimuli of varying degrees of complexity. Animals can perform successfully on DMS without applying a true DMS rule. For example, pigeons may learn to choose accurately in a DMS without being able to apply the response rule to familiar stimuli in an unfamiliar configuration. This suggests that the birds were not matching the stimuli, but had simply learned a number of different conditional responses (Kamil & Sacks, 1972). Similar conclusions have been drawn from work with rats and monkeys (Iversen, 1993; Sidman, 1992). This kind of stratagem would lead to responding at chance levels if novel stimuli were introduced. We therefore assessed performance on DMS using novel comp-objects in a pseudo-trial-unique design, to determine whether the rats had acquired a generalizable DMS rule. Once the rats had learned to perform the task with unfamiliar objects, we introduced the modified forms of boxes into the testing routine. This ensured that any disruption of performance caused by novelty as such would be minimized, making any disruption associated with the form of the stimuli easier to identify in subsequent stages of the experiment.

Method

Phase A: Comp-object boxes. The rats were tested using pseudo-trial-unique procedures for two blocks of 4 days, with 48 comp-object stimuli. Twelve fresh goalbox pairs were randomly selected each day, with the constraint that no goalbox stimulus could be repeated on consecutive days (there were two pools of 24 boxes in use on alternate days). All animals received the same randomized goalbox order and left–right positioning on a given day. Given the reduction in subject numbers after surgery it was possible to increase the total number of trials per day for each rat from 8 to 12. As above, subjects were run as 3 groups of 5 to 6 rats in a discrete trial procedure. Correction procedures were used on the first six trials of each day to avoid the development of side preferences. Testing methods were otherwise identical to those described in the Preoperative Training (Phase 2) section.
Phase B: Introduction of goalboxes of varied complexity. Six novel goalboxes to be used in Experiment 3 were inserted into regular pseudo-trial-unique training as an extension of Phase A. There were 2 days of testing in this phase. All the novel goalboxes were rewarded on the 1st and nonrewarded on the 2nd day of exposure so as to reduce the development of box preferences.

Phase C: Restabilization with comp-objects. Exposure to the goalboxes was followed by another 6 days of training with the pseudo-trial-unique comp-object stimuli used above to restabilize performance.

Results

Phase A. The switch to using pseudo-trial-unique stimuli led to an initial drop in choice accuracy (59.3% overall on the first day), followed by a recovery (88.7% overall correct on the last day). The same pattern of results was seen both in the lesion group and the combined control group. The ANOVA revealed a significant main effect of days, $F(7, 98) = 13.82, p < .005$. There was no effect of lesion, $F(2, 14) = 0.42$, or Days $\times$ Lesion interaction, $F(14, 98) = 0.97$.

Phase B. The introduction of the set of stimulus boxes of varying degrees of complexity led to an overall drop in choice accuracy compared to that seen when testing with only pseudo-trial-unique comp-object boxes. The rats with lesions performed at a higher overall level than the combined controls. Using an ANOVA, we compared performance on the 2 days on which the new boxes were used to performance on the last day before this (in Phase A) and the last day after this (in Phase C), in both of which pseudo-trial-unique comp-object boxes were used. There was a significant main effect of lesion, $F(1, 14) = 10.23, p < .01$; fornix-lesioned group M = 91.2%, control group M = 79.2%. Performance was worse in sessions in which the new boxes were used (81.4%) than in the sessions before and after (89.7%), $F(1, 48) = 7.94, p < .01$: sessions M = 89.7%, novel boxes. The Lesion $\times$ Novelty interaction was not significant, $F(1, 48) = 0.99$.

Phase C. Because choice accuracy had been disrupted in Phase B with a separate ANOVA, we considered the last 4 days of performance with the pseudo-trial-unique comp-objects in order to determine whether performance had restabilized. There was now no significant effect of lesion, $F(1, 14) = 4.17, p > .05$ days, $F(3, 42) = 0.42$, or Lesion $\times$ Days interaction, $F(3, 42) = 1.12, p > .1$. Overall choice accuracy was 92.8%.

Discussion

The poor performance observed at the beginning of Phase A, when the procedure switched from repeated to pseudo-trial-unique testing with comp-objects, probably stems from the tendency to explore novel items, which would lead to incorrect responding on a DMS schedule (cf. Aggleton, 1985). The recovery of choice accuracy might be attributed to the increasing familiarity of the boxes, which would lead to a decline in the exploratory tendency. The rapidity of the recovery (compared to the length of initial training) suggests that the rats had learned a generalizable matching rule during training with a single pair of stimuli (cf. Nakagawa, 1992).

When additional novel boxes were used in Phase B as part of a pseudo-trial-unique procedure, performance was again disrupted although it remained well above chance, reinforcing the suggestion that the rats had learned a generalizable DMS rule. The rats with lesions performed at least as well as the combined controls, and at some points significantly better. At the end of this phase of testing, performance appeared stable and approximately matched, with the lesion group if anything performing better than the combined controls. The good performance shown by the rats with fornix lesions under these conditions and when tested with repeated comp-objects (Experiment 1) provided a suitable baseline from which to assess performance with different forms of stimuli. In Experiment 3, we therefore proceeded to the next stage of testing, in which we used four different kinds of nonspatial stimuli that differed in complexity. The experiment had three phases.

Experiment 3

Method

Phase A. The rats were tested on DMS under five conditions. The first of these was a continuation of the pseudo-trial-unique procedures with comp-objects used in Experiment 2. The other four conditions used repeated goalbox stimuli as follows: (a) new (previously unseen) comp-objects, (b) h/w-objects, (c) shells, and (d) long-simples. These different test conditions were presented in an unpredictable semirandom sequence (adjacent blocks did not start and end with the same condition and the sequence was different in each block) for a total of four 5-day blocks with 12 trials per day. There were thus four determinations of performance under each test condition. Correction procedures were not used during this phase.

Phase B. It became clear in Phase A that the rats in both groups were impaired at DMS using long-simple boxes. Eight days of extra training with long-simple stimuli only were therefore carried out after the end of the fourth block of Phase A. Correction procedures (as described in the Pretraining, Phase 2, section) were used during this extra training period.

Phase C. Following the extra training with long-simple stimuli, there were three additional 5-day blocks of training with different stimuli, exactly as in Phase A. Correction procedures were not used.

Results

Phase A. Analysis of the data from the four blocks of Phase A revealed a highly significant effect of box type, $F(4, 56) = 38.95$, $p < .005$, because both lesioned and control groups failed to acquire DMS with long-simple goalboxes. There was no main effect of lesion, $F(1, 14) = 2.75$, ns, nor a Lesion $\times$ Box Type interaction, $F(4, 56) = 1.42$. There was a main effect of blocks, $F(3, 192) = 3.23, p < .025$, which interacted with lesion in significant Blocks $\times$ Lesion, $F(3, 192) = 3.19, p < .025$, and Box Type $\times$ Blocks $\times$ Lesion interactions, $F(10, 192) = 2.08, p < .05$; these appeared to stem from inconsistent fluctuations of choice accuracy as training progressed.

Phase B. Analysis of the data from the 8 days extra training with long-simple stimuli again revealed no main effect of lesion, $F(1, 14) = 0.90$, but there was a significant main effect of days, $F(7, 97) = 3.30, p < .005$, and a significant Lesion $\times$ Days interaction, $F(7, 97) = 3.96, p < .005$. This arose because the controls rapidly improved on the task, but the fornix-lesioned group did not (see Figure 2). Post hoc
Figure 2. Acquisition of the delayed matching-to-sample task, using repeated presentation of long, simple goalboxes, by the combined control (solid line) and fornix-sectioned (dotted line) groups. The bar to the right indicates 2 standard errors of the difference of the mean for between-groups comparisons, taken from the significant Lesion × Days interaction in the analysis of variance.

Tests showed that the fornix-sectioned group performed nonsignificantly better than the controls, t(97) = 1.94 and 1.70, ps > .05, on the first 2 days, but that the controls performed significantly better than the fornix-sectioned group, t(97) = 2.64 and 2.71, ps < .02 and .01, respectively, on the last 2 days. The fornix-sectioned group did not improve between the 1st and the 8th day of testing, t(97) = 0.34, whereas the controls showed a marked improvement, t(97) = 5.38, p < .002.

Phase C. In a final analysis, we considered the three blocks of testing in this phase. There was a significant main effect of box type, F(4, 56) = 15.31, p < .001, a lesion × box type interaction, F(4, 56) = 3.53, p < .02, and a box type × blocks interaction, F(10, 150) = 2.63, p < .01, but no significant three-way interaction between lesion, box type, and blocks, F(10, 150) = 1.73, p > .05, indicating that performance had stabilized. Post hoc comparisons showed that the fornix-sectioned group significantly outperformed the combined control group when tested with pseudo-trial-unique stimuli, t(56) = 2.08, p < .05, but that this relative superiority was reversed in testing with repeated long-simple stimuli, t(56) = 2.09, p < .05 (see Figure 3). Within-groups comparisons showed that both the fornix-sectioned and the combined control groups performed significantly better with repeated comp-objects than with repeated long-simple stimuli, t(56) = 7.00 and 3.38, respectively, ps < .002.

Discussion

Performance in Phase A of this experiment showed first that the repeated use of stimuli within a test session did not lead to a selective impairment of DMS performance in rats with fornix lesions, and second that the rats in both groups performed particularly poorly with the long-simple goalboxes.

The first of these findings is consistent with the results from Experiment 1 using repeated presentations of comp-objects. However, it extends those results in two ways. First, the present experiment comprises a much larger data set than Experiment 1. Second, and more important, it is now possible to generalize the findings of Experiment 1 from complex objects to the much simpler, transitional forms of stimuli (b/w-objects and shells) used in the present experiment. It thus seems possible that lengthy preoperative training with repeated presentations of complex object stimuli may prevent posttraining fornix section from impairing DMS choice accuracy, even when simple stimuli are used. However it is not possible to extend the generalization to include long-simple stimuli, because both groups performed so badly in this testing condition.

The second finding implies that there is some difference between the long-simple goalboxes and all the other types of goalbox used, including the transitional types. We have previously observed that DMS and DNMS performance in both unoperated subjects and subjects with lesions of the hippocampus or fornix declines substantially when they are first tested with boxes of the long-simple type, following initial training with comp-object boxes (Lyford, Gutnikov, Clark, & Rawlins, 1993; Rawlins et al., 1993; Yee & Rawlins, 1994). In all three studies, control performance recovered as testing proceeded. The two latter experiments included lesion groups; their performance remained poor, despite this recovery of control performance. However, none of these studies used such a mixture of test conditions as was used here, and the present experiment’s lack of differentiation of performance in the two groups over four test sessions with long-simple stimuli probably reflects that aspect of this design.

In Phase B, under conditions in which long-simple boxes were used consistently, the controls showed a clear improve-
ment in performance, whereas the rats with fornix lesions did not. This finding essentially replicated the results of our earlier studies: Lesions of the hippocampus or fornix appear to impair working memory tasks using long simple stimuli. This is not because rats with lesions cannot tell the two boxes apart, because no such impairment is seen when the same two stimuli are used as discriminanda in a discrimination learning task (Rawlins et al., 1993).

In Phase C, we undertook a further direct comparison between the different kinds of boxes. A lesion-induced impairment now appeared, but only when the rats were tested with long-simple boxes. The lesion group actually performed better than controls in the other four of the five testing conditions, and significantly so when tested with pseudo-trial-unique comp-objects. Therefore this result cannot be attributed to a lesion-induced failure to learn the DMS rule. Nor can it be considered to be simply a result of task difficulty, since control performance with pseudo-trial-unique comp-objects was almost identical to control performance with long-simple objects.

The outcome of Phase C was completely unexpected: It demonstrated that the presence or absence of a complex object within the goalbox was not the critical determinant of the working memory deficit that we had previously seen with goalboxes that were relatively plain and differed mostly in surface shade and texture (Lyford et al., 1993; Rawlins et al., 1993; Yee & Rawlins, 1994). Had that been the critical determinant, then the use of shell stimuli in the present experiment should have led to a lesion-induced impairment just like that seen with long-simple stimuli, and the b/w-object boxes might have been associated with an intermediate degree of impairment. Instead, the impairment seemed specific to the long-simple stimuli. In the remaining experiments, therefore, we concentrated on the further analysis of why this class of stimulus poses particular problems for rats with fornix lesions in working memory tasks.

First, we investigated the possibility that some feature of these stimuli leads to enhanced levels of interference (Experiment 4). Our results appeared to rule out any account of this kind. Second, we tested the possibility that the location of the experimenter-specified choice point critically determined the lesion-induced deficit against the possibility that the overall size of the long-simple stimuli was critical (Experiment 5). The results clearly indicated that the size of the goalbox was critical. Third, in Experiment 6, we attempted to reinstate good performance in the lesion group by adding complex objects to long-simple stimuli.

### Table 2

<table>
<thead>
<tr>
<th>Box type</th>
<th>Control group*</th>
<th>Fornix-sectioned group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 4 (SED = ±3.7)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Comp-object</td>
<td>91.9</td>
<td>87.7</td>
</tr>
<tr>
<td>Long-simple</td>
<td>75.0</td>
<td>61.8</td>
</tr>
<tr>
<td>Mixed-pair*</td>
<td>87.5</td>
<td>71.8</td>
</tr>
<tr>
<td><strong>Experiment 5 (SED = ±4.6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short-simple</td>
<td>91.4</td>
<td>84.5</td>
</tr>
<tr>
<td>Long-simple</td>
<td>90.1</td>
<td>73.7</td>
</tr>
<tr>
<td><strong>Experiment 6 (SED = ±6.5)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Object-simple</td>
<td>75.6</td>
<td>57.4</td>
</tr>
</tbody>
</table>

Note: Different pairs of box types were used repeatedly within sessions. SED = standard error of the difference of the mean for between groups comparisons, taken from the appropriate stratum of the analysis of variance. Comp-object = complex object boxes; long-simple = black or white simple boxes; short-simple = shortened black or white simple boxes; object-simple = black or white simple boxes containing objects.

*The control group consisted of both the sham-operated and sham-lesioned control groups. *The mixed pair was a comp-object paired with a long-simple.

in Experiment 3 the rats with fornix lesions chose particularly accurately with comp-object stimuli but particularly inaccurately with long-simple stimuli. Therefore, if the errors seen with long-simple stimuli resulted from an interference effect, the rats should have no difficulty in choosing when faced with a choice between items derived from two different classes.

### Method

Three conditions were run in rotation (one per day) with the following goalbox pairs: (a) a new pair of comp-objects; (b) mixed pairs, in which each trial comprised a choice between a long-simple stimulus and a comp-object; and (c) a fresh pair of long-simple stimuli, constructed to the same specifications as the original pair. The mixed-pair condition used the actual goalbox stimuli used in Conditions A and C, presented in four possible combinations (the long-simple stimulus could be black or white, and could be presented with either of the two comp-object stimuli). Conditions were again presented in an unpredictable semirandom sequence. There were four such 3-day blocks at 12 trials per day. Correction procedures were not used.

### Results

The rats with fornix sections showed impaired choice accuracy when tested either with long-simple stimuli or mixed pairs, but not when tested with comp-objects (see Table 2). The ANOVA revealed a significant main effect of lesion, $F(1, 14) = 13.32, p < .005$, a main effect of box type, $F(2, 28) = 66.42, p < .005$, and a Lesion × Box Type interaction, $F(2, 28) = 5.17, p < .025$. There was also a Box Type × Blocks interaction, $F(9, 135) = 5.39, p < .005$, which arose because overall performance improved significantly across blocks of testing with comp-objects and with long-simple stimuli, minimum $t(135) = 2.46, p < .05$, but not with mixed pairs, $t(135) = \ldots$
The three-way interaction between lesion, box type and blocks, \( F(9, 135) = 1.63, p = .1 \), was not significant.

Post hoc t tests showed no significant difference between fornix-sectioned and control groups with repeated comp-objects, \( t(28) = 1.13 \). The fornix-sectioned group chose less accurately than controls both when tested with repeated long-simple stimuli, \( t(28) = 3.54, p < .002 \), and when tested with mixed pairs, \( t(28) = 4.22, p < .002 \). Within-groups comparisons demonstrated that both groups chose significantly less accurately when tested with long-simple stimuli than when tested under either of the other two conditions, minimum \( t(28) = 3.85, p < .001 \). However, the fornix-sectioned group but not the control group chose significantly less accurately when tested using mixed pairs than when tested using comp-objects, \( t(28) = 6.17 \) and \( 6.61, p < .001 \) and ns, respectively.

Discussion

The results from Experiment 4 demonstrate that rats with fornix sections not only perform poorly on a nonspatial DMS when tested with long-simple stimuli, but also perform poorly when tested with mixed pairs of stimuli. In this condition only one element was a long-simple stimulus; the other was a comp-object. This result rules out the possibility that the deficit seen when rats with fornix sections are tested using pairs of long-simple stimuli stems from within-trial interference from the relative lack of features differentiating long-simple stimuli.

The data were therefore further subdivided to assess choice accuracy in the mixed pairs condition as a function of whether the long-simple stimulus should have been chosen or should have been avoided on the choice run. If long-simple stimuli are somehow less memorable than comp-objects, then one might expect rats to be less likely to choose accurately when the sample was a long-simple box than when the sample was a comp-object. However, the pattern of data obtained was the opposite of this. In both groups, choice accuracy was lower when the sample stimulus was a comp-object, and so the long-simple alternative should have been avoided (77% and 56% for the controls and the lesion group, respectively); these scores are strikingly close to those obtained when the rats were tested with just a pair of long-simple stimuli (see Table 2). When the sample was a long-simple stimulus, so the comp-object alternative should have been avoided, the controls and the lesion group obtained scores of 98% and 88%, respectively. The controls' score in this condition exceeds their score when tested with the comp-object pair; the lesion group's score did not differ at all between these two conditions.

These results indicate that the lesion group was especially defective in avoiding long-simple goalboxes. One possible account of this tendency lies in the different physical location of the experimenter-defined choice point. This lay closer to the beginning of the goal arm when long-simple stimuli were used than when comp-objects, b/w-objects, or shells were used. Perhaps the rats with lesions suffered from disinhibition (Amsel, 1993; Douglas, 1967; Gray, 1982), which would have made it harder to stop running, turn back, and select the alternative goalbox when the designated choice point lay close to the beginning of the goal arm. This possibility was examined in Experiment 5.

Experiment 5

The results from Experiment 3 had shown that fornix lesions selectively impaired nonspatial working memory performance on the long-simple boxes while leaving performance on the empty box shells unimpaired. Neither of these two classes of boxes contained complex objects: Within each class, the two boxes differed from one another only in their paint finish, which was black or white. Thus the same differentiating feature supported good nonspatial DMS performance on days when the rats were tested with shells, but was associated with poor DMS performance on days when long-simple boxes were used.

Despite having identical paint finishes, the long-simple boxes differed from the shells in several respects. First, they were constructed of different materials: plywood versus hardboard, respectively. Second, they differed in overall size, and in details of shape at the back and the sides (see General Method). Third, and as a consequence of the difference in size, the shells were set farther back from the beginning of the goal arms than the long-simples: this was because when the boxes were placed in the maze their rear walls were aligned at the same point in the maze. Because the response criterion was that the rat should place three paws simultaneously on the floor of the goalbox, this criterion was reached nearer the beginning of the goal arm when the long-simple stimuli were used than when the shells were used.

Experiment 5 was intended to demonstrate which of these differences was responsible for the differential lesion effect seen with the different classes of boxes. This was achieved through the use of a new class of goalbox: short-simple boxes.

Method

Apparatus. Short-simple boxes were constructed just like the long-simple boxes, except that the floor was the same length as that in the shells (17.3 cm instead of 25.4 cm; see General Method). These boxes were placed farther forward than the shells had been, so that the front edge of the floor lay at the same point in the maze as the front edge of the long-simple boxes. This meant that the rear end of the short-simple boxes was approximately 8 cm farther forward in the goal arm than the rear wall of the long-simple boxes.

Behavior. There were 8 days (analyzed as four 2-day blocks) of DMS testing using a single pair of goalbox stimuli on each day, alternately a pair of unused long-simple boxes and the new short-simple boxes. The site of food delivery was slightly modified for both sets of stimuli, because the hole above the goal area was too far back to allow delivery to the shortened boxes. Reward pellets for both information runs and correct choice runs were therefore dropped through a gap in the Plexiglas lid: This lay 12.5 cm closer to the start of the goal arm than the original site of food delivery. Correction procedures were not used.

Results

The rats with fornix lesions chose less accurately when tested with long-simple stimuli than when tested with short-simple stimuli; the control rats did not (Table 2). Analysis of variance revealed a significant main effect of lesion, \( F(1, 14) = 6.73, p < .025 \), a significant main effect of box type, \( F(1, 14) = 33.73, p < .005 \), and a significant Box Type × Lesion interaction, \( F(1, 14) = 18.88, p < .005 \). There were no significant effects of
blocks, suggesting that performance was stable. Post hoc t tests showed that whereas the fornix-sectioned group again performed significantly worse than the combined control group with long-simple stimuli, $t(14) = 3.54, p < .01$, they were not significantly impaired with short-simple stimuli, $t(14) = 1.49$. Within groups, the controls performed almost identically with the short and full-length boxes; the performance of the fornix-sectioned group declined significantly when tested with long-simple stimuli, $t(14) = 7.20, p < .002$.

**Discussion**

The results from Experiment 5 were straightforward. Rats with fornix lesions were selectively impaired with long-simple stimuli and showed no significant impairment with short-simple stimuli. These two classes of stimuli were identical in terms of construction material, paint finish, and location of the choice point in the maze. Their general shape was also identical, except that they differed in terms of the length of the floor and the sides. The feature that apparently determines the impairment shown by rats with fornix sections when tested on discrete-trial DMS using long-simple stimuli is therefore simply the length of the goal box. The contents of the goal box seem to play no important part. In a final experiment, we reassessed this last conclusion by testing the rats using long-simple goal boxes into which we had placed complex objects.

**Experiment 6**

**Method**

A further form of modified goal box was introduced for this experiment: long, simple boxes containing objects (object-simples). The simples of Experiment 3 were modified by placing objects near the open end of the goal box (i.e., near the choice point). Familiar objects from comp-objects were chosen for this purpose (the contents of the two boxes used in pretraining and Experiment 1).

There were 5 days of DMS testing, all with object-simples, with food delivered through the hole above the goal area as usual. General procedures were identical to those of Experiments 4 and 5, to allow comparison.

**Results**

Performance in fornix-sectioned and control groups (Table 2) was strikingly similar to that seen in testing with long-simple stimuli in Phase C of Experiment 3 (Figure 3). There was thus no evidence that the introduction of objects into long-simple boxes improved performance in either group. Analysis revealed a significant main effect of lesion, $F(1, 14) = 17.40, p < .005$, because the lesion group (57.4% correct overall) still showed a clear impairment with respect to the combined control group (75.6% correct overall) on this task version. This impairment appeared to be stable: There was no Lesion × Days interaction, $F(4, 56) = 1.23$.

**Discussion**

The results of Experiment 6 support the conclusion reached in Experiment 3 that the presence or absence of complex objects in the goal box makes no difference to the accuracy of discrete-trial DMS performance after fornix section. Even the presence of salient objects near the choice point did not restore the performance of the fornix-sectioned group, despite the good performance that this group had shown when tested using the same objects in the smaller goal boxes used for comp-object stimuli.

**General Discussion**

We have previously shown that, although lesions of the fornix or hippocampus do not necessarily impair nonspatial working memory performance when complex objects are used as the items to be remembered (Aggleton et al., 1986; Mumbly et al., 1992; Rawlins et al., 1993), a clear lesion-induced impairment can be seen when large, relatively simple goal boxes are used instead of objects (Rawlins et al., 1993; Yee & Rawlins, 1994). The present results add to the body of evidence showing that these lesions can lead to performance impairments on DMS (Jagiello et al., 1980; Raffaele & Olton, 1988; Rawlins et al., 1993) and DNMS (Olton & Feustle, 1981; Yee & Rawlins, 1994) tasks that require working memory for goal box or goal-aim stimuli. The presence of a lesion effect does not depend upon the need for animals to use allocentric spatial cues. However, the present results demonstrate a striking limitation on the conditions under which these impairments are seen. The critical determinant of the presence or absence of a lesion-induced impairment was not the lack of clear features (e.g., complex three-dimensional objects) differentiating the stimuli to be remembered: What appeared to be crucial was simply the size of the stimuli themselves. The rats with fornix lesions solved nonspatial discrete-trial DMS only so long as the stimuli were small rather than large.

This conclusion is consistent with the pattern of findings that have previously been reported from rat studies in which conventional lesions of the fornix or hippocampus have impaired (Jagiello et al., 1990; Olton & Feustle, 1981; Raffaele & Olton, 1988) or spared (Aggleton et al., 1986; Aggleton, Blindt, & Rawlins, 1989; Mumbly et al., 1992; Rothblat & Kromer, 1991; Shaw & Aggleton, 1993) nonspatial DMS or DNMS performance. It also may resolve the apparent discrepancy between our earlier positive findings with rats and the negative findings previously reported from a study of monkeys (Correll & Scoville, 1965; see Introduction): Correll and Scoville used stimuli that were small. But why should the size of the stimuli matter in these kinds of tasks? Few theories of hippocampal function offer any account.

One possibility that can clearly be discarded is an interference account. The results from testing with mixed pairs of stimuli are clearly inconsistent with any experimentally testable explanation along these lines, and there are no obvious grounds for supposing a priori that goal boxes that differ simply in size differ in their capacity to generate interference. Within-trials interference can thus be excluded. Moreover, there is evidence that between-trials interference does not make a

---

1 Testing ended at this point because the laboratory closed for refurbishment. It seems possible that, despite the evidence of stable performance, there might have been some improvement in choice accuracy with continued testing (compare with mean scores for Experiment 5).
critical contribution either: Yee and Rawlins (1994) showed that rats with hippocampal dysfunction showed a performance deficit when tested with large, simple boxes in a four-arm radial maze with one trial per day. The same animals showed no deficit when tested in the same way and in the same apparatus but using smaller, complex object boxes. These two procedures did not differ in between-trials interference; they differed only in the nature of the stimuli that were used.2

A second account that seems to have been ruled out is the behavioral inhibition account. The rats with fornix lesions could apparently stop their approach to a first goalbox and switch their approach to the alternative, so long as the first goalbox was not a long one. This ability did not depend on the point in the goal arm at which the rats were deemed to have made a choice; they could do it with short boxes that were set either towards the distal or the proximal end of the goal arm.

Rawlins et al.'s (1993) previous, tentative account of the impairment seen when rats with lesions of the fornix or hippocampus were tested on DMS with long-simple goalboxes suggested that the rats might treat these somewhat two-dimensional simple stimuli as whole environments rather than as discrete, large objects. They suggested that the rats with lesions might find it harder to change arbitrarily the valence of whole environments than to change the valence of discrete objects. This view combined an account of the need for a working memory component in the task with a categorization of critical stimulus types. However, it now seems clear that the simple–complex dimension of stimulus types was wrong: The critical dimension in the present experiment was size.

Our original account could be recast in these terms, but such a move must also raise the possibility of an account in quite different terms; it might be possible to generate some form of alternative, spatial account. An alternative account would need to suggest that a critical, qualitative change occurs as goalboxes increase in size; they change from being discrete objects to being spatial arrays. For a small animal, an item that the experimenter regards as an object might not have to be very large for the animal to treat it as a spatial environment rather than an object. It is interesting to speculate about whether hippocampal "place" cell activity might be seen in long—but not short—simple goalboxes in rats performing our task: Would unit activity follow the behavioral dissociation we have seen? A spatial view might thus suggest that what has previously been presented as a clear nonspatial working memory deficit could instead be described as a spatial working memory deficit, albeit of a very unusual kind in which the places to be remembered constantly switch their relationships to the environment of which they are part. This latter feature should preclude any cognitive map from being formed or used to solve the task (O’Keefe & Nadel, 1978, p. 95).

What alternative kinds of spatial account might there be? It has previously been proposed that the hippocampal may function as a temporary memory store (Rawlins, 1985). This suggestion was cast in terms of the need to store the elements that make up temporally discontinuous associations. In normal rats, the apparent strength of learning decreases as a function of increasing temporal discontinuity (Mahoney & Ayres, 1976; Marlin, 1981; Tanner, Rawlins, & Mellanby, 1987); there is evidence that this decrease can be exacerbated following hippocampal damage (Leaton & Borczsz, 1990; Rawlins, Feldon, & Butt, 1985). The imposition of spatial discontinuities can also weaken learning in normal pigeons (Rescorla & Cunningham, 1979). The critical difference between using short-simple and long-simple goalboxes as stimuli in DMS might thus be described in terms of spatial variation of associative distance. However, this alone is insufficient, because in simple discrimination learning, rats with fornix lesions discriminate between different long-simple boxes as rapidly as controls (Rawlins et al., 1993). The stimulus–response relations required in DMS have to be flexible; when this requirement for flexibility is coupled with a large goalbox, a lesion-induced deficit is seen. It thus seems possible that the rats with lesions fail to use cues flexibly if those cues are associatively distant from the outcome of the response. This is not a mapping hypothesis as such, but if animals were unable to form or use flexible associations of this kind, then they would almost certainly be unable to form or use spatial maps.

Just as it is possible to investigate temporal discontinuity effects by parametrically varying the time between informative stimuli and the outcomes they predict, it is also possible to investigate spatial discontinuity effects by distancing cues from the outcomes they predict. Thus the kind of experimental analysis begun in Experiment 6 might be developed to manipulate the ability of rats with fornix lesions to perform DMS that use complex three-dimensional objects as stimuli. Systematically varying the critical objects' positions within the goal arm might systematically vary task difficulty for the rats with lesions. Such experiments would inform theoretical accounts of the effects of changes in goalbox size demonstrated in the present experiments. It may be that when goalboxes increase in size, they change from being treated as objects to being treated as spatial arrays; but this argument could not be extended to suggest that small objects of a given size turn into spatial arrays when they are located farther from the point of reinforcement. If increasing the spatial discontinuity between objects and outcomes increases the DMS performance deficit induced by conventional hippocampectomy or fornixotomy, then that would provide strong support for an account in terms of associative distance rather than cognitive mapping.

Could other current theories of hippocampal function provide a neater account of our data? The configural association theory (Sutherland & Rudy, 1989) states that trial-unique matching tasks can be solved using a novelty-based simple associative solution that does not depend on the hippocampal system. This readily accounts for the observation that hippocampal dysfunction has no consequence for matching performance.

2 There is a limitation on the generality of our conclusions concerning the effects of box size (pointed out by an anonymous reviewer). Given that the evidence suggests that neither within- nor between-trials interference is a critical determinant of DMS performance after hippocampal damage, our strategy of systematically manipulating box size has so far been restricted to a repeated DMS schedule. However, it is possible that larger stimuli might also be remembered by hippocampal animals if more cues were available and the stimuli did not have to be repeated within any one day. Specifically, we could present goalbox stimuli of different sizes and shapes on a pseudo-trial-unique schedule.
with (pseudo-) trial-unique stimulus presentations (see Sutherland & Rudy, 1989). Sutherland and Rudy proposed that when no novelty strategy is available because stimuli are presented repeatedly, the configural association system would be needed to solve the task; this would require the integrity of the hippocampal system. Earlier findings are consistent with this view (Rawlins et al., 1993): Changing from pseudo-trial-unique stimuli to repeated stimuli of equivalent complexity led to performance deficits in rats with hippocampal lesions or fornix lesions. However, that deficit was not as substantial as the deficit seen when large, simple goalboxes were used repeatedly. Our present results reveal no effects of stimulus repetition as such, only effects of goalbox size (see earlier discussion). We see no way in which the configural account would have predicted these specific effects of goalbox size; however, in interpreting our results post hoc, configural theorists might be able to appeal to the potentially spatial nature of larger boxes. This move would be functionally identical to our own suggestions.

An account that more closely parallels our own interpretation is provided by Kesner (1990, 1991) who proposed that the hippocampus is particularly involved in encoding spatial-temporal information. Kesner states that the hippocampus is not required for encoding visual object-attribute information. This view can therefore accommodate the absence of a lesion effect with smaller boxes, if it is assumed that they are encoded as objects. Aspects of our alternative spatial account of the impairment seen after fornix-section in DMS with larger stimuli seem to fit Kesner’s position on what makes tasks sensitive to hippocampal dysfunction. But our focus on the possible importance of associative distance is more parsimonious than Kesner’s emphasis on the need to encode spatial and temporal attributes as distinct factors that increase vulnerability to the effects of hippocampal dysfunction (Kesner, 1991).

Finally, the proposal that the hippocampus allows the organization of memories according to the relationships between items—the “relational” hypothesis of hippocampal function (Eichenbaum, Otto, & Cohen, 1994)—includes the view that spatial processing puts especially strong demands on relational representation and representational flexibility. The fact that hippocampal dysfunction impairs DMS performance with repeated use of large, but not small, simple stimuli seems to suggest that larger boxes, like standard spatial arrays, increase this need for relational representation and representational flexibility. But the relational hypothesis has nothing to say a priori about the fact that the physical nature of the stimuli determines whether or not DMS is sensitive to hippocampal dysfunction (see Rawlins, Deacon, Yee, & Cassidy, 1994).

It thus seems that current theories of hippocampal function need further development if they are to account for our finding that a small quantitative change in stimulus size is sufficient qualitatively to change DMS performance from being relatively invulnerable to the effects of hippocampal dysfunction to being reliably sensitive to those effects.

References

Murray, E. A. (1992). Medial temporal lobe structures contributing to recognition memory: The amygdaloid complex versus the rhinal...


Received October 18, 1994
Revision received January 11, 1995
Accepted January 13, 1995