The Effects of Hippocampal Formation Ablation or Fimbria-Fornix Section on Performance of a Nonspatial Radial Arm Maze Task by Rats

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Rats with fimbria-fornix section or complete hippocampal aspiration lesions were postoperatively compared to unoperated controls in a nonspatial working memory task using an enclosed plus maze. Initial training (stage 1) was on a continuous two-choice nonmatching to sample task, using pseudo-trial-unique stimuli. Subsequent testing (stages 2–4) always had four boxes available at each choice opportunity, with each goal arm containing a distinctive goalbox. The positions of these boxes were interchanged between choice opportunities, so as to ensure that their relative positions were variable. Extramaze cues were as far as possible excluded. Choices of goalboxes that had not yet been visited within each test session were rewarded. In stage 2, the goalboxes were complex in design, and rats with lesions chose as accurately as controls. In stage 3, within-session interference effects were demonstrated, but these effects were not exacerbated by the lesions. Stage 4 demonstrated that when goalboxes that were simple in design were used the rats in both lesion groups showed a substantial impairment of choice accuracy, even though choice accuracy using complex goalboxes remained unimpaired. This extended previous work demonstrating that hippocampal dysfunction can be associated with nonspatial working memory impairments when large boxes differing in surface texture and appearance are used as stimuli, even though no such impairments are seen when smaller boxes containing complex objects are used. Further experiments will be needed to establish the critical features underlying these nonspatial working memory deficits.

[Key words: hippocampal formation, fimbria-fornix, nonspatial memory, delayed nonmatching to sample task, rat]

There is widespread agreement that the hippocampal formation plays a critical role in normal memory processing. It has been suggested that this role is a highly specialized one, confined strictly to spatial memory (O'Keefe and Nadel, 1978; Nadel, 1991). One strong argument in favor of a spatial theory is that it seems possible, by changing the spatial memory requirement while keeping other task requirements constant, to change the

probability that conventional lesions of hippocampus or fornix will induce a performance deficit. For example, there are many demonstrations of such lesion-induced deficits in spatial working memory tasks in rats (Olton et al., 1978; Olton and Papas, 1979; Rawlins and Olton, 1982; Rawlins and Tsaltas, 1983; Aggleton et al., 1986, 1992), and equally clear demonstrations in monkeys (Parkinson et al., 1988). When the same subjects have also been tested using nonspatial working memory procedures, the nonspatial version of the task has yielded no lesion-induced deficit (Aggleton et al., 1986; Parkinson et al., 1988), even though such versions may be intrinsically harder than the spatial versions for normal subjects to learn (Aggleton et al., 1986). Parallel findings have been obtained with tests of spatial and nonspatial reference memory in a watermaze (Morris et al., 1982).

In apparent contrast to the consistency of the outcomes of spatial memory tests, the findings from nonspatial memory tasks have been variable. Although there are several demonstrations that fornix section or hippocampectomy can impair performance of nonspatial matching or nonmatching tasks (Olton and Feustle, 1982; Raffaele and Olton, 1988; Jagiello et al., 1990), these must be offset against demonstrations that conventional hippocampal system damage alone is insufficient to produce clear impairments in such tasks (Aggleton et al., 1986; Rothblat and Kromer, 1991; Mumby et al., 1992). These apparently contradictory outcomes suggest that the only safe position with respect to the question of whether conventional lesions of the hippocampal formation impair nonspatial memory is one of "not proven." The present study was intended to address this controversy, by investigating the behavioral parameters that are associated with the presence or absence of a lesion effect in nonspatial working memory tasks. It was not designed to identify which component or components of the lesions underlie any effects that might be observed; that would require further, more selective lesion studies.

Recently, we attempted to resolve some of the apparent discrepancies outlined above (Rawlins et al., 1991). We employed a delayed matching to sample (DMS) design, using a large set of complex goalboxes as pseudo-trial-unique stimuli (Aggleton, 1985). Under these conditions, rats with large conventional lesions of hippocampus or fornix showed no significant impairment. An impairment emerged if a single pair of these complex stimuli was used throughout each day's session. The greatest impairment was obtained using a single pair of less complex stimuli, based on those used by Raffaele and Olton (1988) throughout each day's test (Rawlins et al., 1993). Parallel results were obtained when rats given an intraventricular infusion of

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the competitive NMDA antagonist AP5 were tested in the same fashion (Lyford et al., 1993). This pattern of results suggests that rats with conventional hippocampal formation lesions do show a reliable performance impairment on nonspatial DMS tasks when stimuli are used repeatedly throughout a session and, perhaps in particular, when those stimuli are relatively simple in design.

Those experiments could only assess the effects of stimulus *type* (simple or complex) by, at the same time, requiring stimulus *repetition* (reuse of the same stimuli throughout each day's test session). Essentially this is because it is difficult, and maybe impossible, to construct a large set of simple stimuli so that they can be used in a pseudo-trial-unique manner. The present experiment was therefore designed to address directly the issue of stimulus type. This was achieved by assessing the effects of hippocampectomy and of fornixotomy in a nonspatial four-arm radial maze procedure. The rats were required to remember either goalboxes containing complex objects (Aggleton, 1985), or relatively simple arm inserts (Olton and Feustle, 1981). If stimulus type is a critical determinant of a lesion-induced deficit in nonspatial working memory, as our recent results suggest (Lyford et al., 1993; Rawlins et al., 1993), then the lesions should produce an impairment in the present experiment only when simple goalboxes are used. However, there are other possible reasons for the difference between the positive outcome that Olton and Feustle (1981) obtained and the negative outcomes reported elsewhere (Aggleton et al., 1986; Rothlat and Kromer, 1991; Mumby et al., 1992). Four of these are also addressed in the present experiment.

First, Olton and Feustle's (1981) design required the rats to remember at least two, and perhaps three, nonspatial cues simultaneously in order to make an accurate fourth choice. Hippocampectomy may to some extent affect nonspatial delayed nonmatching to sample (DNMS) tasks when lists of items must be remembered (Steele and Rawlins, 1993). The present experiment provided the same memory load as Olton and Feustle's (1981) experiment. Therefore, if "list length" is a critical determinant of a nonspatial memory deficit induced by hippocampal dysfunction, then the rats in the lesion groups in the present experiment should show a deficit regardless of goalbox stimulus type.

Second, in conventional DNMS tasks the only way that a rat can become familiar with a given nonspatial item is by visiting it on the sample trial. In the radial arm maze, in contrast, all the items are available on every choice, so that it is possible to see an item, and so become familiar with it, without actually choosing that item. On subsequent trials, the rat might therefore need to discriminate between those items that appeared familiar because they had been visited (which should thereafter be avoided) and those items that appeared familiar because they had simply been seen (which should therefore be selected on some subsequent choice trial). It has been proposed that hippocampal system dysfunction can prevent animals from discriminating on this basis (Gaffan, 1985). This possibility was assessed in the present experiment. We used new goalboxes to replace, between choices, all those goalboxes that had not yet been chosen, thereby eliminating this source of potential interference. If hippocampal dysfunction induces a selective inability to discriminate between items on the basis of whether or not a response has been made to those items, then the replacement procedure should selectively improve the performance of the rats with lesions. This procedure in addition forced the rats to use retrospective

memory only, ensuring that they needed to remember lists of three items to choose accurately on the fourth choice.

Third, Olton and Feustle (1981) used the same four boxes as stimuli on every test session, whereas current nonspatial DNMS procedures typically use trial-unique, or at least a substantial number of different pseudo-trial-unique, stimuli. We have already shown that the presence of a hippocampal lesion effect in nonspatial DMS tasks can depend on whether stimuli are used repeatedly within sessions. It is thus possible that the use of the same stimuli throughout testing could underlie the lesion-induced deficit reported by Olton and Feustle (1981). We therefore assessed performance on sessions using the same four simple stimuli every day and compared it to performance on sessions using the same four complex stimuli every day. Moreover, there were two daily test sessions (one of each kind), so as to ensure direct comparability of the outcomes. If stimulus set size is the critical determinant of a lesion-induced impairment, then the rats with lesions should show an impairment regardless of the stimulus type of which the set is composed.

Finally, recent experiments using nonspatial DNMS have investigated the effects of conventional hippocampal formation lesions, while Olton and Feustle (1981) used fornix lesions. In the present experiment, therefore, as in our previous experiment (Rawlins et al., 1993), we compared groups with both kinds of lesions to unoperated controls. We did not include cortical control groups, because the ancillary damage entailed in making the two experimental lesions differs substantially; a common lesion effect could not, therefore, be readily attributed to non-specific ancillary damage.

Materials and Methods

Subjects

Twenty-one male rats of the pigmented DA strain were obtained from Bantin & Kingman (Hull, UK), and were maintained on ad lib food and water until they weighed between 200 and 235 gm, at which time surgery was carried out. They were randomly allocated to fimbria-fornix (FORN, $n = 7$), hippocampal (HIPP, $n = 7$), or unoperated control (UNOP, $n = 7$) groups. All rats were caged singly in the same room throughout the experiment with the temperature maintained at 22°C and on a 12:12 hr light/dark cycle (lights on at 07:00). The rats were food deprived overnight before surgery. Following surgery, the rats in the two lesion groups were given free access to food for a period of 15 d for the HIPP group and 8 d for the FORN group, after which they were gradually introduced to a regime of 1 hr access to lab chow per day. Water was freely available. Postoperative behavioral testing commenced 4 d later. The unoperated control group was introduced to food deprivation 6 d earlier. The rats were weighed every 2 weeks to ensure that their body weight did not fall below 80% of ad lib values.

Apparatus

Four-arm radial maze. The aluminum maze was designed to enhance the salience of intramaze stimuli and reduce the salience of extramaze cues. The angle between adjacent arms was 90°. Each arm was 13.5 cm wide with walls 18.5 cm high. The end of each goal arm was open, to permit the insertion of goalboxes, making a total goal arm length of 34 cm. The central area was square, with sides of 17.5 cm. There was a guillotine door at the entrance to each arm. All the arms and the center compartment had removable tops made of Plexiglas onto which one-way reflective film was applied to minimize the rats' ability to see out. The maze was illuminated by 3 × 2.8 W bulbs mounted near the end of each goal arm. The testing room itself was otherwise not lit.

Complex stimuli. Twenty pairs of hardboard boxes served as the intramaze stimuli that would distinguish each goal arm in the radial maze. The appearance of the boxes in each pair was made as similar as possible, but each pair was distinct from every other. This distinction was achieved by painting the walls and floors of the boxes in different colors and patterns and lining the floors with various materials, such as sandpaper, wooden strips, metal, and so on. In addition, each pair

contained an identical object, such as a soft drink can, metal bracket, or wooden block, and no two pairs contained the same object. Each box measured 12 cm wide and 15 cm high, and the length of the floor was 17 cm. Reward pellets (45 mg, Campden Instruments, UK) could be dispensed into the box via a funnel inserted through the Plexiglas cover of the goal arm.

Simple stimuli. Four wooden inserts whose surface texture and color conformed to the descriptions of Olton and Feustle (1981) served as the simple stimuli. Each of the inserts contained a distinctive set of visual and tactile stimuli on the walls and floor. These were (1) wire netting (square size = 1 cm²) on an unpainted rough wood surface, (2) white paint on a rough wood surface, (3) black and white stripes, each 1.3 cm wide, perpendicular to the long axis of the box, with a groove 0.6 cm deep occurring with each black stripe, and (4) a silvery diamond pattern painted on a rough wood surface. The back wall and side wall were 14.5 cm and 10 cm high, respectively, the long axis of the floor measured 24.5 cm in total length, and the boxes were 12 cm wide. A food well, 2.5 cm in diameter and 0.6 cm deep was mounted on the floor, 1 cm from the back wall.

Procedural control

A BBC-B (Acorn, UK) microcomputer provided on-line assistance to determine the order in which complex stimuli were used during the first stage of training and, during later stages, to determine the positions of the four stimuli when these were interchanged between choices. The microcomputer also acted as a timer for intertrial intervals, and was used to record the animals' responses onto magnetic disks for later analyses.

Surgery

Hippocampal aspiration. Rats ($n = 7$) were anesthetized by intraperitoneal injection of Avertin in a dose of 10 ml/kg. (Avertin consists of 100 gm of 2,2,2-tribromoethanol dissolved in 62 ml of tertiary amyl alcohol; this concentrate is then dissolved in absolute alcohol and 0.9% saline in a ratio of 1:4:50 by volume, respectively.) They were then placed in a stereotaxic headholder and the scalp was cut and retracted to expose the skull. A dental drill was used to make an opening exposing the cortex overlying the dorsal hippocampus. The rats were transferred to a specially designed headholder (Rawlins and Bennett, 1982) and had the dura sectioned and the necessary neocortex removed to allow the hippocampus to be aspirated. All aspirations were carried out under visual guidance, using a Wild M650 operating microscope. The wound was packed with sterile gelatin foam, soaked in sterile 0.9% saline. Sulfonamide powder was applied before the skin was sutured with Michel clips, and 40,000 U of Bicillin (Brocades, UK) was injected intramuscularly.

Fimbria-fornix lesion. The rats in the fornix-fimbria lesion group ($n = 7$) were anesthetized as before, and placed in a Kopf stereotaxic instrument, with skull flat between bregma and lambda. The right temporal muscle was retracted, and a hole drilled through the side of the skull. A fine watchmaker's forceps, specially ground and held horizontally on the stereotaxic manipulator with its tips 1.5 mm apart, was inserted at 1 mm posterior to bregma, with its lower tip 5.3 mm deep from bregma, and moved using the screw drive to a point in the contralateral hemisphere 4 mm past the midline, as defined by the position of bregma. After insertion, the forceps was clamped with a screw, held shut for 2 min, and then opened and retracted. Sulfonamide powder was sprinkled on the wound, the skin was sutured, and Bicillin was injected intramuscularly.

Procedure

Handling and familiarization with test apparatus. The day after arriving in the laboratory, the rats were handled as squads of three for 10–15 min on each of 5 d. Shortly before surgery, there were 4 d of familiarization with a maze different from but resembling the test apparatus. On the last 3 of these days, reward pellets were available, scattered on the floor of the maze. The first surgeries took place 2 d later.

Stage 1: DNMS training with complex nonspatial stimuli. Following recovery from surgery, the rats were trained in a two-choice continuous DNMS task. The procedure was adopted from Aggleton et al. (1986) but was implemented on the four-arm radial maze instead of a Y-maze. The radial maze was used in effect as four overlapping T-mazes, such that only three of the four arms were used in any given trial, one serving

Figure 1. Schematic diagram of continuous delayed nonmatching to sample training procedure. *A*, Initial arrangement of goalboxes, with rat starting in featureless box, choosing between two boxes with identical features for start trial (see Materials and Methods). *B*, First choice trial, starting from goalbox selected on start trial. *C*, Second choice trial, starting from goalbox selected on previous choice trial.

Figure 2. Drawings of hippocampal lesions used in studies. Extent of maximum (left column) and minimum (right column) hippocampal aspiration lesions used in studies is illustrated at three coronal planes of sectioning using drawing tube.

as the start arm with the two adjacent arms acting as choice arms. The rats thus became familiar with the entire maze.

To begin a session, all four doors were closed and the rat was placed in one of the four arms (randomly selected) containing a featureless goalbox. A pair of complex goalboxes with matching features was placed in the goal arms on the left and the right of the start arm. The guillotine doors of the start arm and the two choice arms were then raised and the rat was allowed to choose between the two identical boxes (termed A_1 and A_2 ; see Fig. 1A). A choice was deemed to be made when the rat had placed both front paws on the goalbox floor, whereupon the guillotine doors were lowered. On this start trial the rat was rewarded regardless of its choice. The animal was confined to this box (A_1) for approximately 10 sec, during which time the other two test boxes were replaced. Three guillotine doors were then raised to reveal in one adjacent arm a familiar box that would not be rewarded (A_2 -) and in the other a novel box that would be rewarded (B_1 +) (Fig. 1B). This constituted the first choice trial of a session. After another 10 sec confinement in box B_1 , the second trial began; the animal now chose between box B_2 - and box C_1 +, (see Fig. 1C). This sequence was repeated with new pairs of boxes until 30 min had elapsed or a total of 20 trials (including the start trial) were completed. A microcomputer was used to generate a random order in which to present the 20 pairs of goalboxes. This sequence was also balanced for left/right turns.

If an animal made an incorrect choice one correction trial was run with the same set of goalboxes, and if the animal still failed it was forced to the correct box by closing the guillotine door to the incorrect goal arm. During these correction trials the goalboxes were rearranged so

that entering the positive box required the same body turn as in the test trial. Each sequence of 20 trials were grouped into a block. When a rat reached the criterion of 15 or more correct in three consecutive blocks of 20 trials, it was then moved on to the second stage of the experiment. One rat in the UNOP group never learned to run freely in the maze, and was dropped from the entire experiment.

Stage 2: acquisition of nonspatial four-arm radial maze task. To begin a session, a rat was placed in the center compartment with all the guillotine doors lowered. Four different complex boxes, randomly chosen from the collection of 20, were inserted into the goal arms. For each trial, all the doors were raised and the rat was allowed to go down one of the arms. When the rat had made a choice, all guillotine doors were lowered and the rat was allowed to consume the reward (if it was a correct choice) or was confined for an equivalent period of time before being allowed to return to the center compartment. It was then confined to the center compartment for a further 25 sec, during which period the experimenter interchanged the positions of the four goalboxes. The new spatial configuration of the boxes was random, but with the constraint that it could not involve either a rotation or a reflection of the configuration of the boxes on the previous choice trial. All the doors were raised again and the next trial began. No correction runs were given in this stage. This procedure continued for each session until all four boxes were visited at least once or a total of eight responses were made. There were 30 daily sessions.

Stage 3: replacement of unsampled boxes. This version was the same as that of stage 2 except that after the interchange procedure, those goalboxes that had not been visited during the test session were replaced

Figure 3. Representative fornix-fimbria lesions. Photomicrograph of coronal sections of rat brains stained with Weil's hematoxylin, showing degenerated fimbria-fornix bundle (below) and anterior limit of lesion slightly encroaching on dorsolateral septal nuclei (above).

by novel boxes. Hence, on every trial the box or boxes that would be rewarded if selected could not have been seen on any previous trial. For example, if trial 1 consisted of boxes A+, B+, C+, and D+, and box A was chosen, then on trial 2 the four boxes would be A–, E+, F+, and G+, and the next trial might then be with boxes A–, F–, H+, I+. There were two test sessions each day, one of each version, with the order of testing alternated from day to day. Boxes used in the Repeated version were not featured in the Trial-unique version on that day, and vice versa. Fifteen sessions of each test version were completed.

Stage 4: complex versus simple stimuli. Both versions followed the same general procedure as stage 2. The complex stimulus version was identical to stage 2, except that the same four complex goalboxes were used for every session. The simple stimulus version was identical except that four simple goalboxes were used instead. Due to the longer size of the simple boxes, a choice was considered to have been made when the rat had all four paws on the box floor. Both versions were run every day, as in stage 4, for a total of 20 sessions of each kind.

Histology

At the end of the experiment, all animals were killed with an overdose of pentobarbital (Euthatal) and perfused transcardially with 150 ml of 0.9% NaCl followed by 250 ml of 10% formol saline. The brains were removed and embedded in celloidin, and 50 μ m coronal sections were made, saving one in two through the site of the fornix lesion and one in five through the hippocampal lesion. Sections from the HIPP group were stained with cresyl violet; sections from the FORN were stained with Weil's hematoxylin.

Statistics

All statistical analyses were carried out by the statistical package GENSTAT 5, release 2.1 (Rothamsted, 1990) implemented on VAX/VMS-5, except

that BMDP release 7 was used for the analysis of stage 4 in which trend analyses were of interest.

Results

Histology

Hippocampal lesion. One brain was lost during histological processing. All rats sustained extensive damage to the dorsal hippocampus and dorsal subiculum (see Fig. 2). There was substantial ventral hippocampal damage, except that two rats showed significant sparing of the right ventral hippocampus. One rat had damage that extended into the caudal end of the left ventral subiculum. Cortical damage was restricted to the level overlying the dorsal hippocampus; the corpus callosum was also damaged at these sites. The thalamus was hardly damaged in any case. None of the rats in the HIPP group were excluded from the behavioral analysis.

Fimbria-fornix lesion. All rats sustained complete section of the fimbria-fornix (see Fig. 3). There was cortical damage at the point of entry of the forceps, and there was damage to the caudate putamen along the route of entry. There was limited damage to the posterior, dorsolateral septal nuclei. None of the rats in the FORN group were excluded from the behavioral analysis.

Behavioral

Stage 1: DNMS training with complex nonspatial stimuli. One unoperated animal failed to run in the maze, and was excluded.

Figure 4. Acquisition of nonspatial delayed nonmatching to sample. Histogram shows blocks of 20 trials to reach criterion of rats with fimbria-fornix lesions (hatched column), hippocampal lesions (solid column), or unoperated control rats (open columns). Error bars show 2 SEM.

All the remaining animals reached criterion on the task. The rats in the two lesion groups showed more rapid acquisition than controls, but did not differ from one another. This effect could be seen both in analysis of the number of blocks of 20 trials required to reach criterion (see Fig. 4), and in analysis of choice accuracy over the first seven blocks of acquisition. (The analysis was limited to seven blocks because a number of subjects attained criterion at this point and moved on to the next stage of testing.)

One-way analysis of the number of blocks to criterion revealed a main effect of lesion [$F(2,17) = 4.69, p < 0.025$]. Subsequent t tests revealed that the FORN group differed significantly from the UNOP group [$t(12) = 3.06, p < 0.01$], whereas the HIPP group did not differ significantly from the other two (maximum $t = 1.63$). Two-way analysis of acquisition also yielded a main effect of lesion [$F(2,17) = 6.40, p < 0.01$]. Subsequent t tests revealed that both HIPP and FORN animals chose more accurately than controls over the first seven blocks [FORN, 64%;

Figure 5. Acquisition of nonspatial four-arm radial maze: mean correct choices in first four responses for unoperated controls (open circles) and rats with conventional hippocampus (solid squares) or fimbria-fornix (solid triangles) lesions over 30 sessions of acquisition. Error bars to the right show 2 SEs of the difference of the mean for comparisons between subjects, taken from the nonsignificant two-way interaction in the overall ANOVA: the longer bar is for comparisons between the FORN group and the UNOP group; the shorter bar is for comparisons between the HIPP group and the other two groups.

Figure 6. Choice accuracy with replacement of nonsampled items: mean correct choices in first four responses for unoperated controls (open circles) and rats with conventional hippocampus (solid squares) or fimbria-fornix (solid triangles) lesions over 15 sessions of conventional testing (solid lines) or testing with replacement of goalboxes not yet visited during test session (dashed lines). Error bars to the right show 2 SEs of the difference of the mean for comparisons between subjects, taken from the nonsignificant three-way interaction in the overall ANOVA: the longer bar is for comparisons between the FORN group and the UNOP group; the shorter bar is for comparisons between the HIPP group and the other two groups.

HIPP, 62%; UNOP, 54% (SED = 2.94–3.00)]. The main effect of blocks was also significant [$F(6,102) = 2.38, p < 0.05$], indicating an improvement of performance as a function of training, but there was no lesion $\times$ blocks interaction.

Stage 2: acquisition of nonspatial four-arm radial maze task. One FORN rat was excluded from the experiment because of ill health, reducing the FORN group to six. All the remaining subjects showed a clear improvement in choice accuracy as a function of training. The three groups did not differ from one another. By the end of testing, all three groups had attained a mean choice accuracy of more than 3.5 correct in the first 4 responses, well above the chance level of 2.734 (see Fig. 5).

Two-way analysis revealed a significant main effect of blocks [$F(5,80) = 4.00, p < 0.01$]. There were no significant effects including lesion (both F values < 1.0).

Stage 3: replacement of unsampled boxes. Intertrial replacement of those boxes not yet visited during a given test session led to clear increases in choice accuracy in all three groups. Although this effect was graphically most consistent in the UNOP group, it was apparent in the other two groups as well (see Fig. 6).

ANOVA supported these conclusions. There was a significant main effect of task [$F(1,16) = 12.34, p < 0.005$], and the task $\times$ block interaction also approached significance [$F(2,32) = 2.80, p < 0.10$]. Neither the main effect of lesion nor the lesion $\times$ task interaction attained significance (both F values < 1.0).

Stage 4: complex versus simple stimuli. Choice accuracy in sessions using complex stimuli remained high for all three groups. Choice accuracy in sessions using simple stimuli was at first well below this level; all three groups were equally disrupted by this change in procedure. The rats in the UNOP group showed a

complete recovery to the level of choice accuracy seen in sessions using complex stimuli. The rats in the two lesion groups showed no significant recovery over the entire 20 sessions of testing (see Fig. 7).

A three-way ANOVA confirmed these conclusions. There were significant main effects of task [$F(1,16) = 155.02, p < 0.001$], of lesion [$F(2,16) = 5.02, p < 0.05$], and of blocks [$F(3,48) = 5.58, p < 0.01$]. The blocks $\times$ lesion interaction was significant [$F(6,48) = 2.40, p < 0.05$], and the three-way interaction was also significant [$F(6,48) = 2.77, p < 0.05$; linear component $F(2,16) = 7.16, p < 0.01$].

Discussion

In all the DNMS tasks investigated, rats with conventional lesions of hippocampus or fornix performed as well as, or better than, unoperated controls when complex stimuli were used. The sole task variant on which the animals with lesions performed worse than controls was that using simple nonspatial stimuli rather than complex ones. The two lesion groups never differed from each other. This pattern of outcomes exactly parallels those we have recently reported in discrete-trial DMS tasks (Lyford et al., 1993; Rawlins et al., 1993). The present findings therefore replicate the central findings reported by Olton and Feustle (1981) and demonstrate that these findings are compatible with those of Aggleton et al. (1986), as long as the nature of the stimuli to be remembered is taken into account.

The other features that might have been taken to underlie the apparent conflict between Olton and Feustle's (1981) results and those of Aggleton et al. (1986) did not appear important determinants of the presence or absence of a lesion-induced deficit.

List length can be ruled out, because it was held constant from

Figure 7. Choice accuracy with complex or simple stimuli: mean correct choices in first four responses for unoperated controls (open circles) and rats with conventional hippocampus (solid squares) or fimbria-fornix (solid triangles) lesions over 20 sessions of testing with complex stimuli (dashed lines) or testing with simple stimuli (solid lines). Error bars to the right show 2 SEs of the difference of the mean for comparisons between subjects, taken from the significant three-way interaction in the overall ANOVA: the longer bar is for comparisons between the FORN group and the UNOP group; the shorter bar is for comparisons between the HIPP group and the other two groups.

stage 2 onward, and yet the rats with lesions showed no deficit until, in stage 4, simple stimuli were used.

An inability to discriminate items that have been seen but not visited, from those that have been both seen and visited (Gaffan, 1985), also fails to account for our findings. First, there was no deficit in ordinary testing as long as complex stimuli were used. Second, rats with lesions showed no sign of selectively enhanced choice accuracy when this source of within-session interference was eliminated. Even though this change in procedure clearly increased overall choice accuracy, if anything it improved UNOP performance more than it improved performance in the two lesion groups.

Stimulus set size (exemplified by repeatedly using the same stimuli for all test sessions, rather than selecting a fresh set of stimuli for each day's test) also seemed unimportant. The use of the same four stimuli on every day of testing did not appear to affect choice accuracy as long as complex stimuli were used: performance in stage 3 very closely resembled performance on complex stimulus sessions of stage 4. The deficit seen when simple stimuli were used in stage 4 is therefore a consequence of the stimulus type, rather than the stimulus set size. Last, the rats in the two lesion groups displayed exactly the same patterns of performance throughout.

It is therefore clear that reports of lesion-induced deficits seen

in nonspatial working memory tasks that use simple stimuli (e.g., Olton and Feustle, 1981; Raffaele and Olton, 1988; Jagiello et al., 1990) should not be taken to be inconsistent with reports of no deficit seen in rats with similar conventional lesions of the hippocampal formation or the fornix when tested on nonspatial working memory tasks that use more complex stimuli (e.g., Aggleton et al., 1986; Rothblat and Kromer, 1991; Mumby et al., 1992). The present experiment makes it clear that stimulus type as such is a critical determinant of the presence or absence of the lesion-induced impairment in tasks of this kind. The apparent inconsistencies in the behavioral data thus appear to be resolved, since they have a clearly definable basis in experimental procedure.

We have already proposed that a critical difference between the "complex" and the "simple" goalboxes studied in the present experiments may lie in the fact that the former contained discrete three-dimensional objects, while the latter had somewhat two-dimensional and diffuse differentiating features. We have already discussed this issue at some length (Rawlins et al., 1993). Since the lesions studied here do not impair discrimination learning using either kind of stimulus (Rawlins et al., 1993), it seems that a working memory requirement needs to be combined with the use of relatively simple stimuli if a lesion-induced impairment is to be seen. We therefore proposed that

the more two-dimensional simple goalboxes may be treated as whole environments, rather than being treated as themselves constituting large, discrete objects. We suggested that the rats with lesions may find it harder arbitrarily to change the valence of whole environments than to change the valence of discrete objects (Rawlins et al., 1993). This accounts for the need for a working memory component in the task, as well as addressing the issue of stimulus type. Any resolution of this issue requires further parametric behavioral analysis—for example, varying box size while not varying box complexity. It is of course possible that environments are coded in the same manner as spatial arrays. That raises the issue of which description of the stimuli is the more fundamental, but this is not an empirical issue (Rawlins et al., 1991).

Finally, which aspect of the lesion-induced damage underlies the impairment that we have observed? It has been suggested that deficits in visually guided object matching tasks in monkeys arise from cortical damage in the rhinal area, and not from hippocampal damage as such, even when such damage is combined with amygdalar damage (Zola-Morgan et al., 1989; Gaffan and Murray, 1992; Murray, 1992). The effects previously observed in studies of combined hippocampal and amygdalar lesions (Mishkin, 1978; Saunders et al., 1984) are now attributed to the ancillary rhinal damage caused by the surgical approach to the target areas for the lesion. However, these deficits were seen when memory for complex objects was tested; these are just the conditions under which our lesions produced no deficit. The nonspatial working memory deficit appears only under different conditions. Assuming that findings from monkeys should be consistent with those from rats, our observations with complex objects suggest that the entorhinal or perirhinal cortex must have been functioning normally. This is consistent with our histological findings. It therefore seems parsimonious to assume that the positive results we obtained using simple goalboxes did not result from dysfunction in the entorhinal or perirhinal cortices. However, we do not rule out the possibility that damage to the subicular cortices might be critical determinants of the effects we observed; this could be addressed by further selective cytotoxic lesion studies. Taken together, therefore, our present results remain consistent with our earlier suggestion that combined cell loss in hippocampus proper and in subiculum may be necessary to produce profound behavioral impairments either on spatial or sensitive nonspatial tasks (Rawlins et al., 1991); on this view some aspects of normal working memory performance depend upon normal hippocampal function (see also Lyford et al., 1993).

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