The effects of mammillary body and combined amygdalar-fornix lesions on tests of delayed non-matching-to-sample in the rat

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A series of experiments compared the effects of mammillary body lesions with those of combined damage to the amygdala and fornix on 2 tests of working memory, both of which used the delayed non-matching-to-sample rule. This comparison was based on evidence of the involvement of these regions in anterograde amnesic syndromes. The mammillary body lesions had no effect on the acquisition or subsequent performance of a non-spatial recognition task and had only a mild effect on the acquisition of a spatial forced-alternation task. Although the animals with combined amygdalar plus fornix lesions were able to master the non-spatial recognition task they were impaired when the levels of proactive interference were increased. The same animals were also severely impaired on the forced-choice alternation task. The overall pattern of results is seen as mirroring those found in primates and points to an underlying similarity in the mnemonic roles of these limbic regions.

INTRODUCTION

The delayed non-matching-to-sample (DNMS) task is now established as a standard test of recognition memory. Using this task with monkeys it has been shown that removal of either the hippocampus or the amygdala has only mild disruptive effects on visual or tactile recognition while combined damage to both structures produces a severe, permanent impairment.$^{1,7,22,36}$. Comparable results have been found when major efferent pathways from the amygdala and hippocampus, or their diencephalic targets, are damaged singly or in combination.$^{2,7}$. From these and related findings it has been proposed that the most severe anterograde amnesic syndromes are the consequence of damage to more than one limbic site.$^{17,18}$.

The present study forms part of a series of investigations examining whether limbic structures in the rat brain play a key role in recognition memory and whether, as in the monkey, it is necessary to damage more than one limbic site in order to produce the full deficit. A common feature of these studies has been the use of a one-trial DNMS test of object recognition which borrows many of the features of DNMS tasks given to monkeys.$^{1}$. Studies using this DNMS test have revealed that neither amygdalar nor hippocampal lesions disrupt DNMS performance in the rat.$^{5,6}$. In contrast, combined amygdalar-hippocampal lesions were found to impair per-
formance when either proactive interference or retention delays were increased\(^6\).

The current study sought to re-examine the effects of conjoint amygdalar-hippocampal system damage in the rat by combining amygdalar lesions with destruction of the fornix, the major efferent pathway from the hippocampus. The fornix was selected as it is possible to sever this tract while minimising damage to those callosal and cortical regions involved in aspiration lesions of the hippocampus\(^5,6\). It has already been shown that in monkeys combined amygdalar-fornix lesions severely disrupt DNMS performance\(^7\) and so it was predicted that this combined lesion would produce clear performance deficits in the rat.

The mammillary bodies, major recipients of fornical fibres, have long been linked with diencephalic amnesia in man and other primates\(^9,13,15\). There is, however, a lack of convincing evidence showing that mammillary body damage is sufficient on its own to produce the full amnesic syndrome\(^3,15,35\). It has been argued that diencephalic amnesia, like temporal lobe amnesia, may require damage to more than one site in order to produce the full syndrome\(^18\). Consistent with this is the finding that mammillary body lesions in monkeys produce relatively mild DNMS impairments\(^3,38\), which may only be transient\(^38\). The present experiment therefore compared the effects of combined amygdalar-fornix lesions with those of mammillary body lesions; if the rat mirrors the monkey then the mammillary body lesion will have the less effect. If, however, mammillary body damage alone is sufficient to produce the full diencephalic syndrome then the two groups may be comparable.

In addition to testing object recognition we also examined performance on a spatial forced-alternation task. This spatial test of working memory\(^14,25\), which can also be described as a spatial DNMS task, was chosen as it is highly sensitive to hippocampal system damage\(^28\) and hence would help confirm the effects of the fornical surgeries. This task may also prove sensitive to mammillary body damage as previous studies of rats indicate that mammillary body lesions affect spatial learning tasks\(^30,32,33\). The lesions in the mammillary bodies and amygdala were made with the neurotoxin ibotenic acid. This method helps minimise damage to fibres of passage, an important feature given recent evidence that some "established" consequences of amygdalar damage reflect damage to fibres of passage\(^11\).

EXPERIMENT 1: DELAYED NON-MATCHING-TO-SAMPLE (DNMS)

Materials and Methods

Subjects

The subjects were 28 naive, male rats of the pigmented DA strain (Bantin and Kingman, Hull, U.K.). The rats were kept in groups of 3–5 in standard cages until the start of the study when they were housed individually. The animals were housed in a single room with a 14/10-h light/dark photoperiod, all testing taking place at a regular time during the light period. The animals were fed approximately 15 g of laboratory diet (Labsure ERM) daily so that they did not drop below 85% of normal body weight. At the start of the study the rats were aged about 4 months and weighed between 225 and 260 g.

Apparatus

A full description of the apparatus has been published\(^5\). Briefly, testing was carried out in an aluminium Y-maze, 13 cm wide and 20 cm high. Fifty pairs of hardboard boxes served as both start and goal boxes. These boxes fitted into the end of each arm of the maze forming a total arm length of 26 cm. The appearance of the boxes in each pair was as similar as possible but each pair was distinct from every other pair. This was achieved by painting the boxes and by lining the floors with a variety of different materials. In addition, each pair contained an identical object such as a plastic cup, a metal bracket, or a wooden block, although no two pairs contained the same object. The floors of the boxes, which extended towards the centre of the maze, began 8 cm from a Y-shaped aluminium guillotine door at the centre of the Y-maze. Food pellets (45 mg, Campden Instruments Ltd.) could be dispensed under the back of each box. The Y-maze was...
illuminated by a fluorescent ceiling light 215 cm above the apparatus and the luminant light level in the centre of the maze was 290 lux.

**Behavioural**

The training procedure was identical in every respect to that described in previous related studies. At the start of each session the rat was placed in an arm of the Y-maze which contained a featureless box. The central door was then raised and the animal allowed to choose between 2 arms which contained a matching pair of distinctive goal boxes (A1, A2). Upon entering one arm with all four paws the central door was lowered. On this first run, the animal was rewarded with 3 food pellets whichever box it entered. The animal was confined to this box (A1) for 20 s during which time the other two test boxes were replaced. The central door was then raised revealing a familiar box (A2) in one arm and a novel box (B1) in the other. The animal was rewarded with 3 food pellets if it entered the novel box B1.

After 20 s confinement in box B1 the second trial began. The central door was raised and the animal chose between the now familiar appearance of box B2 (negative) and a novel box C1 (positive). This sequence was repeated with new pairs of boxes for a total of 10 trials during which selection of the novel box was always rewarded (trial 3: C2 vs. D1 + ...; trial 10: J2 vs. K1 + ). A balanced schedule determined whether the correct response was to the right or left. The sequence of test boxes was varied after every 50 trials so that any particular box occurred, on average, in every 5th session. If an animal made an incorrect choice correction trials were run with the same set of goal boxes until the animal selected the novel box.

When a rat reached the criterion score of 40 or more correct responses in 5 consecutive sessions (80%) the 2nd phase of the experiment began. During this 2nd phase each rat received a further 150 trials in which retention intervals of ‘0’ s (training condition), 20 s, and 60 s were imposed. For the two longer retention intervals the animals were once again confined in the start box for 20 s, but the box was then removed and the animal tipped into the arm of the Y-maze. The start box was then immediately replaced by a blank, featureless box with no floor. The animal was not handled during this procedure. Following a further 20 s or 60 s confinement in the arm with this blank box the central door was raised revealing a novel goal box and one which resembled the original start box. As before the animal was rewarded for choosing the novel box and an incorrect choice was followed by correction trials. This procedure meant that the animal had to retain the memory of the start box for at least 20 s or 60 s. The animals received 5 sessions (50 trials) at each of the 3 conditions in a mixed, counterbalanced order.

Immediately after these 150 trials the rats received a further 10 sessions, each session containing 12 trials. Half of these sessions were the same as those during the acquisition phase, session-unique stimuli being used on every trial. But, on alternate sessions the rats were tested with a limited set of just 4 pairs of goal boxes, a different set of 4 pairs being used in each of the 5 sessions. During these sessions each pair was used as the correct ‘unfamiliar’ stimulus 3 times. On the first occasion (trials 1–4) each goal box was indeed unfamiliar, but on trials 5–8 each box had been entered once already while on trials 9–12 each box had been entered twice. Thus a sequence of correct goal boxes over the 12 trials might be as follows; A, B, C, D, A, C, D, B, D, A, C, B. As a consequence it can be seen that on trials 5–12 the animal was required to make a relative recency rather than a recognition judgement. A standard series of 5 different goal box sequences was given to each animal. In all other respects the testing conditions, including correction trials, were precisely the same as in the original acquisition procedure and in the intervening, comparison sessions.

**Surgery**

Each rat was anaesthetized by intraperitoneal injection of either 3 ml/kg of chloral hydrate/pentobarbitol mixture (containing 42 mg/ml chloral hydrate and 9.7 mg/ml Nembutal) or 20 ml/kg avertin (containing 20 mg/ml tribromoethanol and 10 mg/ml tertiary amyl alcohol). The animal
was then placed in a stereotaxic headholder (David Kopf Instruments) and the scalp was retracted to expose the skull. A dental drill was used to make an opening exposing the dura above the target structure.

For those animals receiving mammillary body (MB) lesions a single injection of 0.5 µl ibotenic acid (0.5 mg/100 µl, Sigma), dissolved in phosphate buffer (p.H. 7.2), was made through a 1-µl Hamilton syringe in each hemisphere. Each injection took 5 min and the needle was left in position for a further 5 min before being retracted. The injection coordinates relative to ear-bar 0 with the incisor bar set at +5.0 were: AP +3.0, DV +1.0, LAT +/−0.7. An identical procedure was used for the sham-operated controls (SHAM) except that the needle was only lowered to a height of EB0 +2.5 and then withdrawn immediately.

For those animals receiving a combined amygdalar-fornix lesion (A + F) the radiofrequency fornical lesion was made first. An insulated stainless steel insect pin with a 1.0-mm bare tip was lowered vertically into 2 sites per hemisphere and at each site a current of 20 V/8 mA was passed for 30 s (Grass LM4 lesion maker). The lesion coordinates relative to ear-bar 0 with the incisor bar set at 5.0 were: AP +4.4, LAT +/−0.9 and AP +4.2, LAT +/−1.9. In all cases the dorsal/ventral coordinate was 4.0 mm below the height of the dura. The procedure for the amygdalar lesions was identical to that used for the mammillary body lesions except that 2 sites were injected with 0.6 µl ibotenic acid (0.5 mg/100 µl) per hemisphere. The lesion coordinates relative to EB0 were: AP +5.1, DV +1.6, LAT +/−3.7 and AP +4.1, DV +1.6, and LAT +/−3.8. Upon completion of all of the above surgeries Sulphanilamide powder was applied and the skin sutured.

At the end of the study every rat was perfused intracardially with 5% formal saline. The brains were subsequently blocked, embedded in wax (Paraplast), and cut in 10-µm coronal sections. Every 10th section was mounted and stained with a Nissl stain (Cresyl violet). Every adjacent section was also stained but with a fibre stain (Luxol fast blue). The relative sizes of the lesions were estimated by plotting the area of complete or near-complete neuronal loss onto either 8 (mammillary body) or 5 (amygdala) standard, coronal sections spaced equally through the structure. A planimeter was then used to measure the extent of ibotenic acid damage.

**Results**

**Histological analysis**

The ibotenic acid damage in the MB lesions was always restricted to the mammillary bodies (Fig. 1). In 2 cases, however, the lesions involved less than approximately 35% of the structure and the data from these animals were discarded. In the remaining 6 animals the extent of total cell loss from the mammillary bodies ranged from 60% to 93% (median 80%). In all cases the lesions were symmetrical and centred in the medial nucleus.

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**Figure 1.** Diagrammatic reconstruction of the lesions. Left: the smallest (vertical lines) and largest (cross-hatched) amygdalar lesions, and the extent of fornical damage in the same animals. Right: the smallest (cross-hatched) and largest (vertical lines) mammillary body lesions. The numbers refer to the approximate corresponding AP levels from the stereotaxic atlas of Pellegrino and Cushman.⁷
There was no evidence of cellular degeneration in structures connected to the mammillary bodies, e.g. the anterior thalamic nuclei or the hippocampus. It was evident that the neurotoxin spared the many fibres of passage that pass by the mammillary bodies (Fig. 2).

The amygdalar lesions were consistently placed in the central and medial portions of the structure (Fig. 1). Variations in the extent of the lesions meant that 2 cases with extensive bilateral damage to the pyriform cortex were excluded from the study. In the remaining 8 animals the amygdalar lesions, which damaged from 23% to 77% of the entire structure (median = 43%), involved virtually all of the major nuclei, although the lateral nucleus often showed the greatest sparing. In all but 2 animals there was very slight damage to that portion of the putamen immediately above the central nucleus. In 1 case there was some unilateral involvement of the entorhinal cortex.

The fornical lesions produced very extensive damage to the fibre tract itself and in some cases also involved the rostral head of the hippocampus (Fig. 1). In 3 animals the lesion encroached bilaterally into the most dorsal region of thalamus, but this additional damage did not correlate in any clear manner with the behavioural findings. Fig. 2, which shows 2 representative cases, illustrates how only the most lateral tips of the fimbria were spared.

**Behavioural findings**

Following histological analysis the groups contained 10 SHAM, 6 MB, and 8 A + F animals. All animals reached the 80% acquisition criterion (Fig. 3), with the MB and A + F animals typically requiring fewer trials than the SHAM animals. This acquisition difference was not, however, significant ($F_{2,21} = 2.56$). An additional analysis compared the number of correct responses made over the very first 20 trials (2 days) of the DNMS.
task. This follows from the discovery that normal rats will spontaneously non-match\(^1\). It was found that the score of every animal was above the chance score of 10 (mean, SHAM = 14.0, MB = 11.8, A + F = 13.6; minimum \(t\), \(P < 0.01\)), but there were no group differences (\(F_{2,21} = 2.52\)).

Although there were indications that both the MB and A + F lesions might affect performance when the animals received 50 trials at the 2 longer retention intervals (Fig. 3) an analysis of variance using the results from all 3 delays found no lesion effect (\(F_{2,21} = 1.29\)) nor a lesion \(\times\) delay interaction (\(F_{4,42} = 1.16\)). There was, however, a marked effect of delay (\(F_{2,42} = 14.64\), \(P < 0.001\)). As the performance of the animals on the '0'-s condition was matched, an additional analysis just considered the combined scores on the 2 longer delays, 20 s and 60 s. Once again there was no overall lesion effect (\(F_{2,21} = 2.39\)).

All animals were then tested on their ability to perform the DNMS task at '0' s delay when the goal boxes were repeated within a session. Fig. 4 shows the mean performance of the 3 groups over 40 trials (the last 8 trials from 5 sessions) in which each correct 'unfamiliar' stimulus had already been used within that session (mean percent correct, SHAM 61.5\%, MB 57.5\%, A + F 49.1\%). These scores were compared with 40 equivalent trials from the intervening normal sessions. An analysis of variance confirmed that the animals made considerably more errors when stimuli were repeated within a session (\(F_{1,21} = 78.66\), \(P < 0.001\)). In addition, there was a clear lesion effect (\(F_{2,21} = 8.21\), \(P < 0.005\), reflecting the poor performance of the A + F group (Fig. 4), while the MB group did not differ from the SHAM animals. There was, however, no evidence of an interaction between the

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**Fig. 3.** Non-spatial delayed non-matching-to-sample. Left: mean trials to criterion. Right: performance with increasing retention delays between stimulus presentation and choice test. Vertical bars show standard errors.

**Fig. 4.** Recency test. Left: mean performance over 40 trials in which the stimuli were either session-unique (normal) or repeated within a session (repeated). Right: performance plotted against the number of intervening goal boxes between repetition of a particular goal box. Vertical bars show standard errors.
lesions and the 2 test conditions ($F_{2,21} = 1.09$) even though the group scores on the intervening ‘normal’ sessions did not differ significantly ($F_{2,21} = 2.08$). An examination between amygdalar sparing and total scores on the recency test in the A + F group revealed no clear evidence of a correlation ($r_s = 0.13$).

A more detailed way of analysing the same data is shown in Fig. 4 in which recognition performance is plotted against the number of intervening test boxes between the reoccurrence of the same goal box. The test sequences were arranged so that every rat received a total of 8 trials at each of the different number of intervening boxes $^{1,5,6}$. The results for 10+ stimuli came from the last 8 trials from each of the intervening normal sessions, the total being divided by 5 to make it comparable.

An analysis of variance comparing performance with 1–5 intervening boxes confirmed that, as expected, the task became easier as the number of intervening stimuli increased ($F_{4,34} = 14.43, P < 0.001$). The same analysis showed that there was a lesion effect ($F_{2,21} = 6.63, P < 0.01$), reflecting the poor scores of the A + F group (Fig. 4). There was, however, no evidence of a lesion x order interaction ($F < 1$).

The use of exactly the same apparatus and procedure permits comparison with 2 previous studies $^{5,6}$ in both of which the performance of the control groups over 0, 20, and 60 s did not differ from that of the control group in the present study [$F < 1$ (ref. 5); $F_{1,18} = 1.80$ (ref. 6)]. Individual comparisons between the combined scores for the 20-s and 60-s delay conditions indicated that the scores of the A + F group (mean 70.0%) were lower than those of animals with either hippocampal removals (mean 74%, $t = 1.97, P < 0.05$) or neurotoxic lesions of the amygdala (mean 74.5%, $t = 2.05, P < 0.05$). In contrast, the A + F animals achieved higher scores than a group with combined amygdalar-hippocampal lesions (mean 63.2%, $t = 2.30, P < 0.025$). It may also be added that the scores of the MB animals did not differ from those of hippocampectomized animals ($t = 1.06$), whose performance was seemingly unaffected by the surgery.

Lastly, the results from the repeated test box condition were compared with those from a previous study which had used an identical procedure $^{6}$. This analysis showed that the A + F animals (mean score 49.1%) performed worse than animals with amygdalar lesions (mean score 61.2%, $t = 4.35, P < 0.001$) but did not differ from those with combined amygdalar-hippocampal lesions (mean score 52.8%, $t < 1$).

### DISCUSSION

Rats with mammillary body lesions (MB) or combined amygdalar-fornical lesions (A + F) were tested on a DNMS task modelled on tests of object recognition which have proved sensitive to limbic damage in monkeys $^{17,36}$. The rats were rewarded for selecting the goal box that differed from the start box (non-matching) and as both the start and goal boxes changed after every trial this task taxed working memory $^{14,25}$. Although the task might be regarded as a simultaneous oddity problem, observation showed that the rats very rarely attempted to make a direct comparison between all 3 boxes before making a choice and hence relied on working memory. Evidence that the animals had not used some unintentional cue to solve the task came from the chance performance of the animals on the hardest condition (1 intervening item) of the recency test (Fig. 4).

It was found that MB lesions did not affect post-operative acquisition of the DNMS task, indeed, if anything the task was learnt somewhat faster than normal. These lesions, which removed up to 90% of the neurons in the entire mammillary body region, had no clear affect on performance with longer retention periods (20 s and 60 s) and did not disrupt the recency judgement task. These insubstantial effects are reminiscent of the mild, and possibly transient, effects of mammillary body lesions in monkeys $^{3,38}$.

Like the MB animals, the rats with combined amygdalar-fornix lesions were able to acquire the DNMS task rapidly. Although the performance of the A + F rats over the retention delays was poorer than that of the controls this difference was not significant. Nevertheless, comparisons with previous studies indicated that the scores of the A + F animals were intermediate between
those of animals with either amygdalar or hippocampal lesions, who were better, and animals with combined amygdalar-hippocampal lesions (A + H), who were worse on the delay conditions. In this respect the results are consistent with studies of monkeys which indicate that incomplete damage to amygdalar and hippocampal systems results in an intermediate deficit. Repetition of the test boxes within a session, which taxed recency judgements, produced a clear impairment in the A + F animals, the overall scores not differing from chance. Given that these same animals were able to acquire the DNMS task at a normal rate it is unlikely that this reflects a motivational or sensory deficit. A more likely explanation is that the deficit reflects the change in proactive interference. In the normal DNMS condition the level of proactive interference is set by the fact that each box is re-used every 5 days and by any similarities between different boxes. While these effects may be slight in the standard task, any such interference would be considerably exaggerated in the recency test in which each box was presented 3 times within a single session. It is therefore suggested that the A + F lesions resulted in an increased sensitivity to proactive interference, possibly as a consequence of altered temporal tagging of events. This same explanation was used to account for the similar pattern of deficits observed after combined amygdalar-hippocampal lesions.

EXPERIMENT 2: SPATIAL NON-MATCHING-TO-SAMPLE

Introduction

Experiment 2 examined whether the MB and A + F lesions were sufficient to disrupt a memory task known to be sensitive to hippocampal system damage. The animals were therefore trained on a rewarded alternation task, run as a test of working memory. In this task the animal is forced to enter one arm of the T-maze and then rewarded for running into the other arm. Previous studies have confirmed that this task is highly sensitive to hippocampal or fornix ablations and transection of the mammillothalamic tract.

Materials and Methods

Subjects and apparatus

The subjects were the same as those used in the previous experiment. The floors of the T-maze were 10 cm wide and made of aluminium. The stem of the maze was 80 cm long with a guillotine door located 33 cm from the beginning. The cross piece was 136 cm long and at each end there was a food well 4 cm in diameter and 0.75 cm deep. The walls of the maze were 17 cm high and made of clear Perspex. The maze was supported on 2 stands 93 cm high. Lighting was provided by fluorescent lights suspended 92 cm above the apparatus, the luminant light levels at the choice point and food wells being 320 and 280 lux, respectively.

For the final part of the study an additional cross arm was fitted onto the stem of the T-maze so forming a double T- or I-shape. Both cross arms were 136 cm long and contained food wells 4 cm from the ends, making a total of 4 wells. The central stem was now 97 cm long and a wooden block was used to separate the top and bottom halves of the 2 connected T-mazes. All testing was carried out in a large room different to that used in Experiment 1 in which tables, sinks and empty cage racks provided salient room cues.

Behavioural

Testing began within 25 days of completion of Experiment 1. Each animal only required 1 or 2 days of pretraining in order to run reliably down the stem of the T-maze to find food pellets in both of the food wells.

At the start of each trial, which consisted of 2 stages, 3 food pellets (45 mg, Campden Instruments) were placed in each food well and a wooden block was placed in one arm close to the choice point. On this ‘information run’ the animal was forced by the wooden block to enter the open arm and was allowed to eat the food there. No retracing was permitted. The animal was then picked up and placed back in the start box, and the wooden block removed. On this ‘choice run’ the rat was deemed to have chosen when it had placed a back foot on either choice arm, whereupon the wooden block was placed behind the rat.
to stop retracing. If the rat had alternated, i.e. entered the arm it had not visited on the forced run, it was allowed to eat the food and then returned to its cage. If the other arm was chosen, i.e. the same arm as on the ‘information run’, the rat was confined to the arm for approximately 10 s and then returned to its holding cage. The rats were tested in groups of 3 or 4 with each rat having 1 trial in turn. As a consequence the intertrial interval ranged from 3 to 5 min. The animals received 6 trials each day, each consisting of 2 runs through the maze.

All rats received 12 sessions (72 trials) on this task at which point testing of the A + F animals stopped. The SHAM and MB animals were, however, tested immediately after on 2 more forced-alternation conditions. In the first of these the retention intervals between the ‘information’ and ‘test’ trials were varied. The MB and SHAM rats received 15 sessions, each of 6 trials, in which 3 different intratrial intervals (10 s, 30 s and 60 s) were used in a balanced, mixed order. The rats were placed in their cages during all 3 intratrial intervals. The intertrial interval was from 3 to 6 min.

In the final condition the MB and SHAM rats were tested in the double T-maze. Each rat received 4 double trials per session for a total of 5 sessions (40 trials). Each rat was placed in the central start box and each of the 4 food wells was baited with 3 45-mg reward pellets. On the ‘information’ trial the rat ran to the ‘top’ choice point where it was forced by a wooden barrier to enter either the right (R) or left (L) arm. After the rat had eaten the food reward it was picked up and returned to the start area and now allowed to run to the ‘bottom’ choice point where it was forced into one of the arms. Four different ‘information’ trials were possible i.e. RR, RL, LL, LR, and they were used in a balanced, mixed sequence. The rat was now returned to the start area and allowed to run to the ‘top’ choice point where alternation was rewarded. This procedure was then repeated immediately afterwards for the ‘bottom’ half of the maze. The rats were tested in groups of 3 or 4 and each pair of trials for a given rat was separated by 6–8 min.

Results

Figure 5 shows the mean performances of the experimental groups over successive blocks of 3 test sessions during acquisition. The A + F group showed a severe impairment which was confirmed by the large Group effect when comparisons were made over these blocks of acquisition sessions \( (F_{2,21} = 39.35, P < 0.001) \). There was, in addition, a clear effect of blocks showing that, taken overall, the animals were improving with repeated sessions \( (F_{3,63} = 13.29, P < 0.001) \).

The total scores of the A + F animals were also compared with those of 14 animals with combined lesions of the amygdala and hippocampus and with 8 hippocampectomized animals, all of whom had received an identical training programme. No evidence was found \( (F < 1) \) that the scores of the A + F animals differed from those of 2 additional groups, in both of which the surgery resulted in a complete transection of the fimbria/fornix.

The acquisition scores of the MB group were also lower than those of the SHAM animals and this was confirmed in a separate analysis of variance \( (F_{1,14} = 12.15, P < 0.01) \), although there was no evidence of a session x lesion interaction \( (F < 1) \). There was, however, no clear correlation between total acquisition scores and the extent of mammillary body damage among all 8 animals.

![Fig. 5. Spatial delayed nonmatching-to-sample. Mean performance over 4 successive blocks of 3 test sessions, followed by 3 different retention intervals (10 s, 30 s, 60 s), and the double T-maze.](image-url)
that were tested \((r = 0.29)\). Taken over all 12 sessions the mean percent correct for the SHAM group was 91.9\%, while the MB group averaged 84.7\%, and the A + F group averaged only 58.9\%.

In contrast, the MB group showed no evidence of an impairment when the animals were tested with varying retention delays (Fig. 5) and this was confirmed in an analysis of variance \((F < 1)\). Similarly, there was no evidence that the MB lesions disrupted the ability to remember 2 locations at the same time \((t < 0.1)\).

DISCUSSION

As expected the A + F animals were severely impaired on the spatial alternation task and their scores were close to chance (Fig. 5). This result is consistent with other studies of hippocampal system damage\(^5,16,28\). Furthermore, the performance of the A + F animals did not differ from that of A + H animals in which the hippocampus was removed and the fimbria/fornix completely transected\(^6\). This finding, although limited by floor effects, presumably reflects the extensive fornical damage in the A + F animals.

The results of the MB lesions are of interest as the use of ibotenic acid helped minimise any involvement from those tracts, such as the medial forebrain bundle, that run close to the mammillary body region. The finding of a clear, but mild, deficit in the initial acquisition phase is consistent with a range of other studies showing that mammillary body damage can affect reinforced\(^12,33,34\) and spontaneous\(^8,31\) alternation in a T-maze. The normal performance over the retention delays is also consistent with some\(^33\), but not all\(^31\), previous studies.

One explanation for the effects of mammillary body damage on spatial alternation is that the lesions increase sensitivity to proactive interference\(^8,34\). Accordingly, the acquisition scores of the MB and SHAM animals were considered trial by trial (1–6). This analysis revealed that although there was an order effect \((F_{5,76} = 3.26, P < 0.025)\) consistent with a build up of proactive interference within each session, there was no evidence that the MB animals were differentially affected. It must also be remembered that an increased sensitivity to proactive interference may be a secondary consequence of a different impairment and that Experiment 1 found no evidence of an impairment on the high interference version of the non-spatial DNMS task.

Although the mammillary bodies form one of the major diencephalic targets of the fornix it is clear that the deficit associated with their destruction is far less severe than that observed after fornix transection. This conclusion comes from the results of previous studies on the effects of fornix transection on reinforced alternation\(^16,28\) and from a consideration of the A + F animals in the present study. This in turn points to the contribution of other fornical targets in the performance of this spatial working memory task. Candidate regions, along with the mammillary bodies, include the anterior thalamic nuclei\(^30\) and the septum\(^28\).

GENERAL DISCUSSION

The present study forms part of a series of investigations into the involvement of limbic structures in recognition memory. One goal of which has been to determine the degree of similarity between those neural systems involved in recognition in the rat and monkey. To this end previous studies have looked at the effects of hippocampal, amygdalar, and combined hippocampal-amygdalar lesions and have revealed at least a superficial correspondence between rats and monkeys.

The present study found that combined amygdalar-fornix lesions did not affect acquisition of the DNMS task. This is consistent with the effects of combined amygdalar-hippocampal lesions in rats\(^6\) and of similar combined lesions in monkeys when, as in the present study, the retention delay in acquisition is kept very short\(^26,29\). These findings all emphasize the involvement of non-limbic structures in the 'procedural' aspects of the learning task.

A comparison of the retention scores (20 s and 60 s delays) following a variety of lesions reveals a progressive decline in performance [overall; sham 73.8\%, amygdala 74.5\%, hippocampus
74.0%, A + F 70.0%, A + H 63.2%, (refs. 5 and 6) which mirrors that observed in monkeys. One obvious difference, however, is that the effects of combined limbic lesions in rats appear much less severe than that observed in monkeys. This may point to a real species difference. Unfortunately, there are other discrepancies, such as the levels of baseline performance, the extent and nature of the lesions, and the class of stimuli, which must at present weaken any firm conclusions. There is, for example, evidence that damage to adjacent cortical regions may contribute to the severity of the combined limbic lesion effect in monkeys, a factor which has not been systematically examined in experiments with rats although there was no evidence in the present study of a correlation between incidental cortical damage and performance in the A + F animals.

The finding that A + F, as well as A + H, lesions disrupt DNMS when it taxes recency rather than recognition shows that this impairment was not the consequence of damage to non-limbic regions. Furthermore, as previous studies have shown that this deficit is not observed after either amygdalal or hippocampal lesions, and unpublished evidence strongly indicates that fornicotomy is not sufficient to produce this deficit (Shaw and Aggleton, unpublished findings), it would appear that it is the result of conjoint damage in these two regions.

The most comparable data from studies with monkeys concerning the recency task comes from experiments using the delayed matching or non-matching-to-sample task with a small set size. Studies using this procedure indicate that removal of the amygdala impairs performance but that hippocampal damage has little if any effect. Of particular interest is the finding that combined damage produces an even greater impairment. Unfortunately, extended training with a very small set size is likely to encourage the use of an associative strategy i.e. select (avoid) the stimulus most strongly associated with reward. This strategy would, in turn, account for the apparent involvement of the amygdala in recency judgements. In contrast, the rats in the present study were transferred suddenly onto the recency task and so were unlikely to adopt an associative strategy.

Although fornical lesions were used as a means of assessing hippocampal system damage it must be emphasized that destruction of the fornix and the hippocampus proper are not equivalent. Indeed, there is evidence that under certain circumstances fornictomy in monkeys may increase responsiveness to novelty and even significantly improve DNMS performance. Furthermore, fornix lesions in rats can significantly improve the acquisition of the DNMS task (Shaw and Aggleton, unpublished findings). This may in turn be one of the reasons why some of the effects of the A + F lesions in the current study appear to be relatively mild. It should lastly be noted that the effects of fornical damage in rats and monkeys have been found to be qualitatively similar on the spatial non-matching-to-sample task used in Experiment 2. The finding indicates that similarities in limbic function may extend to spatial as well as non-spatial tests of recognition.

The present study found evidence that mammillary body damage can disrupt a spatial memory task and yet leave a non-spatial task unaffected. This selective pattern of dysfunction is quite different to that observed in human anterograde amnesia in which both spatial and non-spatial memories are affected. Furthermore, the mammillary body deficit on the spatial task could only be regarded as mild when compared with that seen after hippocampal damage. The present pattern of results shows that the mammillary bodies are not necessary for a range of mnemonic functions and that, unless there is a marked species difference, this finding is at odds with the belief that mammillary body damage represents the core lesion in human diencephalic amnesia.

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REFERENCES


