The effects of fornix and medial prefrontal lesions on delayed non-matching-to-sample by rats

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INTRODUCTION

The delayed non-matching-to-sample task (DNMS) has become one of the benchmark tests for assessing memory deficits in animal models of amnesia. This behavioural test of object recognition memory also provides a non-spatial test of working memory. As a consequence, DNMS, and the closely related delayed matching-to-sample (DMS) task, have been of particular interest to those investigating the functions of the hippocampal formation. Studies assessing the sensitivity of these tasks to hippocampal system damage in rats have, however, produced conflicting results. The purpose of the present study was to determine whether these inconsistent results arose from differences in the relative familiarity of the stimuli used in the various studies. An additional goal was to measure the effects of medial prefrontal lesions on DNMS acquisition and performance. The rationale for including animals with medial prefrontal lesions arises both from evidence of the involvement of this region in DNMS performance by monkeys\(^3\) and from evidence concerning the role of the medial prefrontal cortex in recency judgements by rats\(^1\)\(^7\).

Evidence that removal of the hippocampus does not disrupt DNMS acquisition or performance in rats comes from a study by Aggleton et al.\(^3\). Rats were trained in a Y-maze to select the goal box that differed from the start box. Aspiration lesions, which removed nearly all of the hippocampus, were found to have no effect on the rate of acquisition or on subsequent tests involving retention delays of up to 60 s\(^3\). In this study a pool of 50 different stimuli were used so that any particular stimulus appeared, on average, once every five sessions. Very similar results have since been reported in two other studies examining DNMS performance in rats with either hippocampal lesions\(^23\) or fornix lesions\(^24\). Both studies used a large pool of junk objects.

In marked contrast, Olton and Feustle\(^25\) found that fornix lesions severely impaired the re-acquisition of a DNMS task in a cross-shaped maze. In this task the animal was rewarded for selecting only those arms that had not been chosen during that session, each of the arms being identified by a distinctive insert\(^25\). Spatial cues were excluded by moving the arms between every
trial, the same four inserts being used on every session. A similar, severe deficit was observed in rats with fornix transections on a test of delayed matching-to-sample. In this test the animal had to return to the goal box it had entered on the sample run, just two distinctive goal boxes being used throughout. Further evidence that hippocampal damage can disrupt matching-to-sample comes from a study using a modified cross-maze. It was found that animals with hippocampal lesions were unable to relearn to select the choice arm (black or white) that matched the sample arm (black or white). This deficit was found even when there was no apparent retention delay.

One possible explanation for these inconsistencies concerns the numbers of test stimuli used in the various studies. While those tasks that found little or no hippocampal deficit used 50 or more test stimuli studies reporting clear hippocampal deficits used only two or four stimuli throughout. This is potentially a very important difference as it changes the demands of the task from a test of recognition between a familiar and an unfamiliar stimulus, to a test of recency between two extremely familiar stimuli.

The impact of this change was tested in the current study. Rats with fornical lesions received 12 trials a day on the DNMS task of Aggleton, but in this study the same set of 13 different pairs of stimuli (12 trials) were used in every session. 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 two 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 sample run, the animal was rewarded with three 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. On the test run the animal was rewarded with three 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 box B2 (negative) and a novel box C1 (positive). This sequence was repeated with new pairs of boxes for a total of twelve trials during

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

Materials and Methods

Subjects

The subjects were 30 naive, male rats of the pigmented DA strain (Bantin and Kingman, Hull, UK). 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 (Beekay Rat and Mouse, Bantin and Kingman, Hull) daily so that they did not drop below 85% of normal body weight. At the start of the study the rats were aged about 3 months and weighed between 225 and 260 g.
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. 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. Each pair of boxes was encountered once per session and the same set of boxes was used for each session. The order of the individual pairs of boxes was, however, changed after every session.

When a rat reached the criterion score of 46 or more correct responses in five consecutive sessions (76.7%) the testing procedure was modified. For the following ten sessions the goal box was removed just prior to the rat making a choice between the familiar and the unfamiliar box and replaced by a blank start box. The rat remained in the maze throughout this procedure. This prevented the rat from making a simultaneous discrimination and so ensured reliance on working memory. This procedure was used for every trial throughout the rest of the experiment.

Recency judgements. For the following ten sessions the number of different test boxes was reduced from twelve to six but each box was now presented twice per session. The second exposure always occurred on trials 7 to 12. The order of the boxes on trials 7 to 12 was arranged so that either 2, 5, or 8 trials intervened between the first and second presentation of a particular box. The order of the individual boxes on trials 1 to 6, and hence 7 to 12, changed every day.

This 'six pair' condition was followed by ten sessions in which just four pairs of test boxes were used throughout. The number of intervening trials between the first and second presentation of a particular stimulus now ranged from 1 to 5. As in the 'six pair' condition, the different numbers of intervening stimuli occurred in equal frequencies. Five 'normal' sessions, in which the full set of 12 test boxes were used, were interleaved between the ten days of the 'four pair' condition.

A further ten sessions were then given using only three pairs of boxes. The numbers of intervening trials were now 1, 2 and 3, each occurring in equal frequency. Five 'normal' sessions were again given at regular intervals throughout this condition.

Finally, two sessions were run in which a completely novel set of 12 test boxes was used (24 trials).

Surgery

Each rat was anaesthetised by intraperitoneal injection (4 ml/kg) of a solution containing 42 mg/kg of chloral hydrate and 9.7 mg/kg of pentobarbitone sodium (Equithesin). The animal was then placed in a stereotaxic headholder (David Kopf Instruments, Tu-

junga), the scalp retracted to expose the skull, and a small craniotomy made above the target structure.

For those animals receiving fornix lesions (FX) an electrode (stainless steel insect pin), insulated except for the tip (1.2 mm) was lowered vertically into two 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. An identical procedure was used for the sham operated controls except that the probe was lowered to just above the fornix and then immediately withdrawn with no current being passed.

Medial prefrontal cortex lesions were made by aspiration using a 23 gauge needle attached to a SAM 12 aspirator (Aerosol Products Ltd., London). The lesions, which were carried out under visual control, started at Bregma and extended for 5 mm anteriorly and included an area 1.5 mm on each side of the midline. The wounds were packed with sterile cotton (Allen and Hanbury, London) soaked in physiological saline. Upon completion of all of the above surgeries Sulphadimidine 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 tenth section was mounted and stained with a Nissl stain (Cresyl violet) and every adjacent section was stained with a fibre stain (Luxol Fast blue).

Results

Histological analysis

Following histological analysis one animal in which half of the fimbria/fornix was spared in one hemisphere was excluded from the FX group. The remaining FX animals all received extensive, bilateral damage to the fimbria/fornix and only the most lateral tips of the fimbria were frequently spared. Fig. 1 depicts the extent of damage in those cases with the largest and smallest extent of fimbria/fornix damage. In all animals the damage also involved the rostral head of the hippocampus (Fig. 1). In two animals the lesion encroached into the most dorsal region of the thalamus, and in one case with a complete transection of the fornix there was some slight additional medial septal damage.

Three animals who received prefrontal cortex surgery displayed only very minor damage in the target area and hence were excluded from the study. On the basis of the
histology the remaining twelve rats were divided into two groups of six, a Large Frontal (LF) and a Small Frontal (SF) lesion group.

The rats in the Large Frontal group had extensive, bilateral damage to cingulate cortical areas 1, 2 and 3 with slight sparing in some animals of the most anterior and posterior portions (Fig. 2). All rats had additional damage to frontal areas 1 and 2^3 and in most cases the lesion extended into the anterodorsal margin of the medial orbital area. The corpus callosum was transected in all animals and in three rats the most rostral portion of the septum was damaged although this did not relate to performance.

Rats in the Small Frontal group (Fig. 2) had damage to cingulate areas 1, 2 and 3 but this was not as extensive as in the LF group, the greatest sparing being in area 3. Additional damage was found in frontal area 2 in all animals and in one rat the lesion encroached, but did not transect, the corpus callosum.

There was no evidence of retrograde degeneration in the dorsomedial thalamic nucleus in any of the rats with medial prefrontal lesions.

**Behavioural findings**

Following histological analysis the groups contained 9 SHAM, 8 FX, 6 SF and 6 LF animals.
DNMS acquisition

All animals reached the acquisition criterion with the FX animals typically requiring fewest sessions (Fig. 3 (upper)). Although this acquisition difference was not significant ($F_{3,25} = 2.15$), there was very clear evidence that the FX animals performed better than the SHAM animals in the early acquisition stages (Fig. 3 (lower)). This difference was confirmed in a comparison of the total number of correct trials made during the first ten sessions (120 trials). The highly significant Group effect ($F_{3,25} = 22.81, P<0.001$) reflected the superior scores of the FX animals. Subsequent comparisons confirmed that the FX group was better than each of the other groups (Newman-Keuls, all $P<0.05$).

In view of this striking result the initial scores of the FX and SHAM groups were re-analysed to determine if spatial factors could have influenced accuracy. This was achieved by identifying those trials in which the correct response involved returning to the arm that the animal had come from on the previous trial (i.e. RL or LR consecutive choices — ‘same’ arm response). These were compared with those trials in which the correct response required the animal to select the third arm, that is the arm the animal had not just come from (i.e. RR or LL consecutive choices — ‘different’ arm response). The percent correct on entering the ‘same’ (70% of all trials) and ‘different’ (30% of all trials) arms was calculated for the first ten sessions of the acquisition phase and for the ten ‘normal’ sessions that were interleaved during the ‘4 pair’ and ‘3 pair’ conditions (Fig. 4).

As can be seen from Fig. 4 (upper), the SHAM rats began with a clear bias against returning to the arm in the place they had just come from i.e. they performed...
worse on 'same' trials. Following acquisition this bias was lost. In contrast, the FX rats did not start with this spatial bias and so performed better than the SHAM rats in the initial acquisition sessions. This pattern of behaviour was reflected in the significant interaction \((F_{1,15} = 5.44, P < 0.05)\) between the factors Group, Type of trial (‘same’ or ‘different’) and Stage of learning (initial or final). Additional paired comparisons helped to confirm that this interaction reflected the abnormal pattern of performance by the FX group (FX vs. SHAM \(F_{1,15} = 5.84, P < 0.05\)). Neither the LF nor the SF groups differed from the SHAM animals during either the first ten or the last ten normal sessions \((P > 0.05)\).

In view of the abnormal pattern of responding shown by the animals with fornix lesions we re-analysed the data from a similar study of DNMS performance by rats with hippocampal aspiration lesions. Accuracy for ‘same’ and ‘different’ arm choices was examined for the first 120 trials (12 sessions) of acquisition and for the 150 trials that immediately followed the acquisition criterion (0, 20 and 60 s retention delays). As in the present study, the pattern of choices made by the control and hippocampectomized animals differed (Fig. 5).

Not only was there a Group \(\times\) Type of trial interaction \((F_{1,17} = 8.99, P = 0.008)\) but there was also a significant Stage of learning \(\times\) Type of trial interaction \((F_{1,17} = 4.71, P = 0.045)\), consistent with the disappearance of a spatial bias once the animals had reached the acquisition criterion (Fig. 5).

**DNMS performance**

Following acquisition all of the FX, LF, SF and SHAM animals were tested on their ability to perform the DNMS task when the number of goal boxes used within a session was systematically varied. While it was found that the various conditions affected performance in a predictable way, there was little evidence that the groups differed from one another.

Comparisons between the 10 sessions used for each of the three successive conditions (‘6 pair’, ‘4 pair’ and
Fig. 5. Delayed non-matching-to-sample. Data taken from Aggleton et al. comparing animals with hippocampal lesions and operated controls. Performance has been divided between those trials where, in order to make a correct choice, the animal was required to enter the arm it had started the previous trial from ('same'), and those trials where the correct choice was to the third arm i.e. to a different location ('different'). The figure shows data for the first 12 sessions of the acquisition phase (120 trials) and the last 15 sessions (150 trials) of the delay condition (retention delays of 0, 20 and 60 s).

"3 pair") confirmed that performance declined with decreasing number of stimuli ($F_{2,50} = 6.71, P = 0.003$) i.e. with increasing proactive interference. There was, however, no evidence of a difference between the groups ($F < 1$). A more detailed way of analysing the same data is shown in Fig. 6 in which recognition performance is plotted against the number of intervening goal boxes ('lag') between occurrence and re-occurrence of a particular goal box within a session. That increasing the 'lag' made the task easier was confirmed in separate analyses of variance ($P < 0.001$ for all three conditions). The same analyses showed that there was no lesion effect ('6 pair', $F_{1,25} = 4.71, P = 0.04$; '3 pair', $F_{1,25} = 1.37$) and no evidence of an interaction ('6 pair' and '3 pair', $F_{3,25} = 1.43$).

Performance on the '4 pair' and '3 pair' conditions was also compared with the five normal sessions that occurred during each of these conditions. These normal sessions could be regarded as setting a baseline from which to compare the more difficult, repeated stimulus versions of the task. Analyses of variance confirmed that the animals made considerably more errors when stimuli were repeated within a session ('4 pair', $F_{1,25} = 116.31, P < 0.001$; '3 pair', $F_{1,25} = 239.41, P < 0.001$). There were, however, no significant differences between the overall levels of performance of the four groups, and the only significant Group x Session Type interaction occurred because the SF group performed particularly well on those sessions of the '4 pair' condition with repeated stimuli.

In the final two sessions, in which a completely novel set of stimuli were used, all groups performed considerably better than chance (mean score from 24 trials, FX 21.6, SHAM 20.3, LF 19.0, SF 18.7). A comparison between the groups just failed to reach significance ($F_{3,25} = 2.84, P = 0.058$) and a Newman–Keuls test showed that no two groups differed significantly from each other at the 0.05 level.

**Discussion**

Rats with fornical lesions (FX) or medial prefrontal lesions (LF and SF) were tested on a DNMS task in which they were rewarded for selecting the goal box that differed in appearance from the start box. For all but the initial acquisition trials the demand on working memory was increased by the removal of the start box prior to the animal making a choice. Levels of performance were then systematically altered by repeating stimuli within a session and by changing the interval be-
tween the re-occurrence of the same stimulus within a session.

Contrary to predictions about the involvement of the hippocampal system in working memory, it is evident that fornical lesions did not impair acquisition or subsequent performance of the DNMS task. Indeed, there was clear evidence that animals with fornical damage performed better than normal on the initial stages of this task. Subsequent analyses showed that this facilitation in non-matching could be explained by the lack of a spatial bias that disrupted normal initial performance. It should, however, be stressed that this bias had disappeared in the control animals by the time they had reached the acquisition criterion, so that subsequent performance can be regarded as comparable. In these subsequent trials there was no evidence that fornix damage interfered with the ability to perform the non-matching task, even when the task was made considerably more difficult by repeating stimuli within a session.

The possibility that animals with fornix damage may have a greater preference for novelty than normal rats, independent of any spatial bias, cannot be completely discounted. It is, for example, possible that the fornix lesions reduced neophobia and so aided the animals in the initial learning phase. Support for this notion comes from evidence that hippocampal damage may reduce neophobia for novel foods (refs. 2, 14, 18, but see ref. 37) and for novel objects placed in a familiar environment. Furthermore, in a recent study of the relationship between the re-occurrence of the same stimulus within a session, even when the task was made considerably more difficult by repeating stimuli within a session.

The finding by Mharzi et al. should, however, be treated with some caution as the test stimuli (one familiar, one novel) were both placed in new locations. As a consequence, the control rats may have been encouraged to explore the familiar object as it was now in a different place. More convincing evidence that fornix damage may increase responsiveness to novelty comes from the finding that monkeys with fornix transections have an enhanced preference for both visual and auditory novel stimuli. This enhanced preference may sometimes aid DNMS performance (see Expt. 6 of ref. 45). Thus, while the most parsimonious explanation for any increased preference for novelty following fornix damage is the indirect result of a spatial impairment, it is possible that an enhanced preference may reflect additional changes.

The effects of medial prefrontal damage upon DNMS performance do not appear to have been examined before in the rat. The present study found no evidence that medial prefrontal aspiration lesions disrupted performance, even under conditions of increased proactive interference. This result appears consistent with the finding that while damage to the closely related medial dorsal nucleus of the thalamus may retard DNMS acquisition in rats, it does not affect subsequent performance. The present finding does, however, suggest that the reported deficits in recency discrimination following medial prefrontal damage in rats may be restricted to tests of spatial memory.

Expt. 2 examined whether rats with fimbria/fornix or medial prefrontal lesions would be disrupted on a memory task known to be sensitive to hippocampal system damage. The animals were therefore trained on a spatial alternation task (spatial non-matching-to-place), run as a test of working memory. In this task the animal is forced to enter one arm of a T-maze and is then rewarded for choosing the other arm. Previous studies have confirmed that this task is highly sensitive to hippocampal lesions.

EXPERIMENT 2: SPATIAL NON-MATCHING-TO-SAMPLE

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 two 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.

Behaviour

Testing began within 25 days of completion of Expt. 1. Each animal required only 1 or 2 days of pre-training 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 two stages, three food pellets (45 mg Campden Instruments, Loughborough) 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 confined in.
the start box for 20 s before the wooden block was 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 one trial in turn. As a consequence the intertrial interval ranged from 3 to 5 min. The animals received six trials a day, and a total of 12 sessions (72 trials).

Results

As expected, animals with fimbria/fornix lesions were severely impaired while those animals with medial prefrontal lesions showed evidence of a slight, but significant, deficit. In the case of the SF group this impairment appeared to be only transient.

Fig. 7 shows the mean performances of the experimental groups over the 12 sessions, blocked into pairs. A comparison across these training blocks revealed a highly significant Group effect \( F_{3,25} = 53.94, P < 0.001 \), reflecting the fact that the FX group were significantly impaired relative to all other groups (minimum \( F_{1,125} = 35.91, P < 0.001 \)). In addition, both the SF and LF groups were impaired relative to the SHAM group (SF vs. SHAM, \( F_{1,13} = 11.17, P = 0.005 \); LF vs.

SHAM, \( F_{1,13} = 8.02, P = 0.014 \)). The finding of a Group \( \times \) Block interaction \( F_{15,125} = 2.12, P = 0.013 \) was consistent with the transient nature of the deficit in the SF group (Fig. 7), this group showing the greatest improvement across the experiment.

GENERAL DISCUSSION

Rats with either fornix or medial prefrontal lesions were tested on two working memory tasks, one spatial the other non-spatial. Both groups displayed impairments on the spatial test (delayed non-matching-to-place) but were unimpaired on a delayed non-matching-to-sample task. The difference in performance levels, which was particularly striking in the FX animals, cannot be attributed to the relative difficulty of the two tasks. The findings for the FX group, which appeared similar to the effects of hippocampectomy\(^3\), provide another example of how hippocampal dysfunction need not honour the distinction between working memory and reference memory\(^5\).

The finding that rats with medial prefrontal cortex lesions were impaired on the spatial task was consistent with previous studies\(^7,39\), as was the transient nature of the deficit in the SF group\(^6,38\). Furthermore, the difference between the LF and SF groups agrees with other studies indicating that these deficits are dependent on the size of the lesion\(^9,36,38\). In particular, it has been suggested that prefrontal impairments on delayed alternation performance depend on the involvement of the prelimbic sector of prefrontal cortex\(^6\). This portion of the prefrontal cortex, which lies in front of the anterior cingulate region, receives many fornical projections arising from the hippocampal formation\(^16\). In contrast, the anterior cingulate cortices receive only very light inputs\(^16\). It is therefore quite likely that the greater impairment observed in the LF group reflects the greater involvement of prelimbic cortex.

A major goal of the present experiments was to resolve apparent differences concerning the effects of hippocampal system damage on non-spatial tests of working memory. While a number of studies indicate that hippocampal or fornical ablation can severely disrupt ‘non-spatial’ tests of matching or non-matching\(^13,25,28\) other studies have failed to find any evidence for such deficits\(^3,21,23,34\).

It is important, at the outset, to identify those tasks that have a spatial component that might confound the results. As has already been explained, the study by M’Harzi et al.\(^21\) should probably be excluded as responsiveness to the novel objects may have been influenced by the novelty of its location. Similar problems arise in a test of stimulus recognition devised by
Markowska et al. in which the animal has to respond to the change of a particular object in a particular place. In spite of these problems both studies do, however, show that animals with hippocampal system damage are sensitive to the introduction of a novel stimulus.

A similar conclusion comes from studies looking at the responsiveness of animals with hippocampal lesions to the arrival of a new stimulus in a test cage or in a runway. The latter study is of particular relevance as it shows that while animals with hippocampal damage can be sensitive to new stimuli they are insensitive to spatial changes in familiar stimuli. This finding serves to reinforce the need to dissociate changes in spatial novelty and object novelty. That this can be a difficult problem to overcome is highlighted by the present study where it was found that normal rats had a spatial bias that influenced initial performance. It is, however, important to note that this bias was lost with training and did not affect the tests with reduced stimulus set size.

The difficulty of excluding spatial factors also arises in the study by Jagielo et al. In this study rats with hippocampal lesions were unable to re-acquire a matching-to-sample task using just two different stimuli (black arm, white arm) in a modified cross maze. Indeed, these rats were unable to re-acquire the task even when there was no retention delay. This latter finding was used as evidence against the ‘working memory’ hypothesis. A detailed consideration of the task does, however, suggest that spatial factors could influence performance. In particular, the position of the maze remained constant within a session. As a consequence rats could aid performance by learning the position of those adjacent arms that formed correct pairs on that session. Unfortunately, the effect of moving the maze within a session was never reported. It is also the case that the hippocampal surgeries sometimes produced extensive cortical damage and this may have influenced performance.

From a consideration of other ‘non-spatial’ tests of working memory it is apparent that one potentially important variable is the distinction between recognition and recency memory. This variable was examined systematically in the present study by altering the number of test stimuli used in the DNMS task. The results were very clear. Rats with fornix lesions were able to demonstrate accurate non-matching with as few as three stimuli per session and with a ‘lag’ of only one (Fig. 6). Thus there was no evidence that fornix damage increases sensitivity to proactive interference. The results of the present study are also consistent with those from a similar DNMS task run in a Y-maze. In that study it was found that rats with cytotoxic lesions of the hippocampus were able to perform normally, even though they had been trained throughout with only three different pairs of boxes (black, white, black and white stripes). It should, however, be noted that unlike the present study the rats received an interval between each trial of approximately 12 min. It has also been reported that rats with extensive entorhinal lesions, involving parts of the subiculum, are able to acquire a working memory task in a cross maze. As the task, closely modelled on that of Olton and Feustle, used only four stimuli it provides further evidence that hippocampal system damage need not disrupt recency judgments between highly familiar stimuli.

In view of the clear evidence that differences in the numbers of test stimuli, and hence levels of interference, will not account for the ‘inconsistent’ effects of hippocampal system damage on DNMS and DMS it is necessary to consider other explanations. One possibility is that those tasks that have revealed hippocampal deficits depend on conditional discriminations. Another possibility concerns the nature of the stimuli used in the matching/non-matching tasks and the way in which these may make different configural demands on the subjects.

It has been pointed out that those matching or non-matching tasks that use the same, small set of stimuli on every session may become conditional discrimination tasks. That is, rather than learning to select the most recently visited box in a matching task the rat instead learns a conditional rule e.g. if black sample box choose black test box. Such a rule is only likely to be learnt when a small number of stimuli are used. Assuming that hippocampal damage does disrupt the ability to use conditional rules this explanation could account for the severe deficits with small set sizes and the normal performance with larger set sizes.

The one apparent exception, a study by Sutherland and MacDonald which used three stimuli throughout and yet found no hippocampal deficit, can be explained by their use of very long intertrial intervals which allowed the rats to perform the task as a genuine recognition test.

This conditional discrimination account is also consistent with the present results. It can be argued that the initial training with session-unique stimuli forced the rats to use a non-matching rule and so prevented the subsequent use of a conditional strategy, even when the numbers of stimuli were reduced. Confirmation that the rats did use a non-matching rule comes from their ability to transfer immediately to a new set of novel stimuli.

There are, however, a number of possible problems with this conditional rule explanation. In particular, the study by Rasmussen et al. which found no deficit on...
the acquisition of a non-spatial working memory task used only four stimuli throughout. This study did, however, focus on the effects of entorhinal rather than hippocampal lesions and this may be an important difference. A more serious problem for the conditional learning explanation arises from evidence that either fornical or hippocampal lesions need not impair the acquisition of conditional discriminations by rats, even when they are spatial. It would appear, therefore, that the evidence for the proposal that the key feature is the use of a conditional rule remains intriguing but unproven.

Another possibility concerns the type of stimuli used in the various experiments. It has been pointed out that those studies reporting hippocampal system deficits have tended to use stimuli that are distinguishable because of differences in surface texture or paint finish (e.g. black vs. white). In contrast, studies finding no hippocampal deficit have frequently used three-dimensional 'junk' stimuli. To test the hypothesis that a difference in stimulus type is important Rawlins et al. trained rats with either hippocampal or fornical lesions on a DMS task using two stimuli. With complex stimuli of the sort used by Aggleton there was a small, but reliable, effect of the lesions on acquisition. This impairment became much more pronounced, however, when stimuli of the kind used by Raffaele and Olton were given.

This fascinating result may be related to the notion that the hippocampal formation is required when the animal must learn to use a relationship among common cues in order to solve the task, the assumption being that highly distinctive junk objects preclude the need for such configural cues. A closely related proposal derives from evidence that monkeys with fornix lesions are impaired at discriminating between complex scenes with common elements. Gaffan has proposed that when each scene (stimulus) can readily be identified by distinct, unique features fornix damage will have no apparent effect. In contrast, fornix lesions will produce an impairment when common elements are repeated so that the animal is required to remember the spatial organization of the scene. Thus, by providing junk objects with many discriminative features rats may be able to perform normally on DNMS tasks, while other less varied stimuli may produce impairments. A problem with this explanation is that it does not predict why an animal with hippocampal system damage should have difficulty distinguishing a white from a striped goal box in one study, but not in another.

From a consideration of the above it becomes evident that different tests of stimulus recognition or recency can not be regarded as equivalent tests of non-spatial working memory. Differences arise in the demand on recency judgements and in the possible application of conditional discrimination strategies. These, in turn, are likely to be influenced both by the number of test stimuli and the length of the inter-trial interval. In addition, the composition of the test stimuli often differs and this may have important consequences. Finally, it must be remembered that all tasks contain a potential spatial element. In some instances this spatial component is quite apparent but in many cases it is far more subtle. For example, if the animal can use spatial cues to help distinguish a sample trial from a test trial then an animal with hippocampal damage may be disadvantaged.

While it must be desirable to find a single explanation for the varied effects of hippocampal system damage on non-spatial tests of DNMS and DMS, this does not yet seem possible. Although the present study has demonstrated that changes in proactive interference are not sufficient to account for the apparent inconsistencies in the literature, there remain a number of other possible causes. It now remains to examine these possibilities in a systematic manner and to identify the critical hippocampal contribution. It is, however, clear that the growing number of examples of hippocampal damage failing to disrupt recency/recognition judgments provides convincing evidence against strong versions of the 'working memory' hypothesis and the 'temporary memory store' hypothesis.

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