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Lesions of the fornix but not the amygdala impair the acquisition of concurrent discriminations by rats

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Rats with lesions in either the fornix, the amygdala, or both were compared with control animals on the acquisition of three different concurrent object discrimination tasks. In the first task the animals received one trial per day on each of six pairs of stimulus objects ('spaced' condition). In the second task the animals received four trials per day on each of six stimulus pairs ('standard' condition), and in the last task the animals received 36 trials on each of two stimulus pairs in just a single day ('massed' condition). Animals with fornical lesions were impaired on all three conditions. In contrast, the amygdala lesions only affected the 'massed' condition and then only when the animals had to select the 'non-preferred' stimulus. Although animals with combined amygdala and fornical lesions were impaired on all three conditions there was no evidence that their deficit was greater than that in the animals with lesions restricted to just the fornix. In view of the evidence that concurrent discrimination learning offers an appropriate test for anterograde amnesia these findings are seen as consistent with the notion that the hippocampus, but not the amygdala, is critically involved in the mnemonic processes disrupted by amnesia.

INTRODUCTION

It has consistently been found that humans with anterograde amnesia are impaired on the acquisition of concurrent visual discriminations. A clear deficit is found irrespective of whether the discriminative stimuli are three-dimensional objects³⁸, photographic slides⁵, or computer-generated symbols¹³. Furthermore, this deficit appears to be independent of the type of feedback used to indicate correct responses^{13,38}. As impairments have been found in subjects with a variety of different brain pathologies, including Korsakoff's syndrome^{5,19,31,38}, postencephalitis¹, and anoxia³⁸, this task seems to provide a sensitive assay for both temporal lobe and diencephalic amnesia.

These findings have helped lead to the suggestion that concurrent discrimination problems should be used as benchmark tests for the assessment of experimentally induced amnesias in non-human primates⁴². In support

of this proposal is evidence that medial temporal lesions, involving both the hippocampal formation and the amygdala, produce marked acquisition deficits in monkeys^{10,11,42}. In these concurrent learning studies the animals are presented with pairs of discriminative stimuli (typically between six and eight) several times a session over a number of sessions. Further experiments using this standard procedure have indicated that damage to the hippocampal formation may be sufficient to impair acquisition^{21,28,42,43}, but that there may be important contributions from adjacent perirhinal and parahippocampal regions⁴⁴.

While concurrent discrimination problems appear to provide a sensitive test for anterograde amnesia in primates, remarkably little is known about the value of these tests for assessing memory impairments in rats. The present study therefore examined the performance of animals with lesions of the fornix, the amygdala, or both, on the postoperative acquisition of three different concurrent discrimination tasks. The effects of fornix transections were examined in the light of evidence of hippocampal involvement in this task in primates.

While fornix transection cannot be regarded as identical to hippocampectomy, this selective surgery does, in the rat, results in very many similar impairments. Animals with amygdala lesions were included as several current theories of amygdala function make different predictions concerning the effects of amygdala damage on concurrent discrimination learning^{2,9,18}. Lastly, animals with both amygdala and fornix lesions were included as it has been proposed that combined damage to the amygdala and hippocampal systems is required to produce the full-blown anterograde amnesic syndrome^{7,23,25}. For this reason it might be predicted that combined limbic lesion should produce the most severe impairments on the learning of concurrent discriminations.

Three different concurrent object discrimination tasks were used in this study. In the first task ('spaced') the animals received only one trial on each of six pairs of objects per day. This follows from the seemingly paradoxical finding that combined removal of the amygdala and hippocampus has no effect on the learning of concurrent discriminations by monkeys when each trial for a given pair of stimuli is separated by 24 h^{22,34}. This result, which is in dramatic contrast to those from other concurrent discrimination problems, may be analogous to some of the spared learning abilities in human amnesics^{25,26}. The first part of the present study therefore set out to determine whether this remarkable example of preserved learning is also found in rats. The second task ('standard') was based on the typical concurrent discrimination design used with monkeys. In the present task, each session consisted of four trials on each of six discriminations. The third condition ('massed') was designed to match the situation in nearly all tests of concurrent discriminations given to humans in which the discriminations are learnt within a single session. For this the rats were only given two concurrent discriminations but they received a total of 72 trials within a single day.

MATERIALS AND METHODS

Subjects

The subjects were 45 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) 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 224 and 265 g.

Apparatus

A Grice box was used for all three concurrent tasks. This fan-shaped box consisted of a small rectangular start box (13 × 18 cm) which was separated from a triangular test area by a guillotine door. The far wall of the test area was 43 cm long and 43 cm from the guillotine door. Two equal sides, 46 cm long, connected this far wall to the entrance of the start box. The walls of the apparatus, which were made of aluminium, were 24 cm high. The plastic floor contained two circular food wells, 2.5 cm in diameter and 35 cm from the start box. An aluminium partition which protruded 16 cm from the middle of the far wall ensured that the rats could not run directly between the two food wells, which were 21 cm apart. The luminance at the food wells was 110 lux.

The food wells, which were made of white plastic, contained numerous fine holes and directly underneath each well was placed a tray containing reward pellets. This arrangement, which was designed to stop the animals from using olfactory cues in solving the discriminations, meant that the rats could not see the hidden pellets but could presumably smell them. Thus, in the event that the presence of food could be detected even when covered by an object, both the positive and the negative food wells would smell similar.

The six pairs of stimuli used for the 'spaced' condition consisted of different metal objects (maximum height 68 mm) attached to various metal bases, all of which completely covered the food wells. Several of the objects were painted in different patterns of black and white. The six pairs of stimuli for the 'standard' condition were all made of painted wood (maximum height 93 mm) and set on bases of different shapes. Care was taken to make the individual stimuli as distinctive as possible, both from one another and from those used for the 'spaced' condition. The two pairs of stimuli used in the 'massed' condition were made from different shaped pieces of wood (maximum height 68 mm), each painted in a different manner. Multiple copies of all three sets of stimuli were used and all of the objects were sealed with coats of clear lacquer paint to help eliminate olfactory cues.

Behavioural

Pretraining began a minimum of 14 days after surgery. During pretraining the animals were trained to run from the start box to find food pellets (45 mg, Campden Instruments Ltd., Loughborough) in either food well by pushing aside a flat, circular wooden disc.

This pretraining procedure was followed by the 'spaced' concurrent discrimination task. In this task each animal received the same six pairs of stimuli, always in the same order, but for only one trial a day. Four reward pellets were placed under the positive stimuli which were determined by the animal's choice on the very first session. The left-right position of the stimuli varied according to a random schedule. A choice was defined as moving a stimulus object (with front paws or snout) and animals were returned to the start box before they could explore the other stimulus in a pair. The animals received one session a day, 5 days a week, until they had either received a total of 60 sessions or had reached a criterion of 16 correct choices in three consecutive sessions (88.9% correct).

The 'standard' concurrent task began 10 days after completion of the 'spaced' concurrent task. The procedure was identical to the previous task except that each session now consisted of 24 trials, made up of four consecutive repeats of the six pairs of discriminative stimuli. Three rather than four pellets were used as reward in order to avoid satiety. The pellets were placed under the positive stimulus of each pair, which was determined by a randomized allocation. While the order of the stimulus pairs was kept constant for all animals, the left-right positions of the stimuli were varied. All animals received 36 test sessions.

Two weeks after completion of the 'standard' task the rats received a final concurrent discrimination test ('massed'). In this test the animals had only two pairs of stimuli, which were used on alternate trials, and only two reward pellets were placed under the correct objects. Testing took place over two sessions, each of 36 trials, separated by an interval of 2 h. By baiting both stimuli on Trial 1 and neither stimuli on Trial 2 of the first session, it was possible to select a 'preferred' and a 'non-preferred' positive stimulus i.e. the one that had been chosen on Trial 1 and the one that had not been chosen on Trial 2, respectively.

Surgery

Each rat was anaesthetized by intraperitoneal injection of 3 ml/kg of a chloral hydrate-pentobarbital mixture ('Equithesin') containing 42 mg/ml chloral hydrate and 9.7 mg/ml Nembutal. The animal was then placed in a stereotaxic headholder (David Kopf Instruments, Tujunga), the scalp retracted, and a small craniotomy made to expose the dura above the target structure.

For those animals receiving amygdala lesions (A), a single injection of 0.39 μ l of an 0.12 molar solution of *N*-methyl-D-aspartic acid (Sigma Chemical Co. Ltd., Poole), dissolved in phosphate buffer (pH 7.2), was made through a 1 μ l Hamilton syringe in each hemi-

sphere. 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 = 4.6, HT = 1.6, LAT = \pm 4.0.

The surgical procedure for the fornical (F) lesions was similar to that for the amygdala lesions except that the lesion was made by radiofrequency. A radionics TCZ electrode (0.3 mm tip length and 0.25 mm diameter) was lowered vertically into the fornix and the tip temperature raised to 72 °C for 60 s using an RFG4-A Lesion Maker (Radionics Inc., Burlington). Two lesions were made in each hemisphere. The stereotaxic coordinates of the lesions relative to ear-bar 0 were: AP = 5.3, HT = 7.1, LAT = \pm 0.7, and AP = 5.3, HT = 7.1, LAT = \pm 1.6.

For those animals receiving a combined amygdala-fornix lesion (A + F) the procedures were identical to those described above, the fornical lesion being made first. The surgeries for the sham amygdala (ASHAM), sham fornix (FSHAM), and sham amygdala plus fornix (A + FSHAM) animals matched those used for the actual lesions except that the needle or radiofrequency probe was lowered to a depth 1.5 mm above that used for the actual lesion and then withdrawn. 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% formol saline. The brains were subsequently blocked, embedded in wax (Paraplast), and cut in 10- μ m coronal sections. Every tenth section was mounted and stained with a Nissl stain (cresyl violet).

RESULTS

Histological analysis

Amygdala (A). Two of the animals in the A group were found to have asymmetric surgeries and their results were excluded from all subsequent analyses. The largest and smallest of the lesions in the six remaining A cases are shown in Fig. 1. In three of the surgeries the neurotoxin produced an almost complete loss of cell throughout the amygdala, although in the three remaining cases there was some unilateral sparing of parts of the basolateral nuclei (Fig. 1). Extra-amygdaloid damage was confined to parts of the piriform cortex (one case) and to the most ventral portion of the putamen, immediately above the central nucleus (four cases).

Fornix (F). In three of the animals with fornical lesions the surgery spared the lateral third of the fimbria/fornix bilaterally and the results of these animals were therefore discarded from the study. The lar-

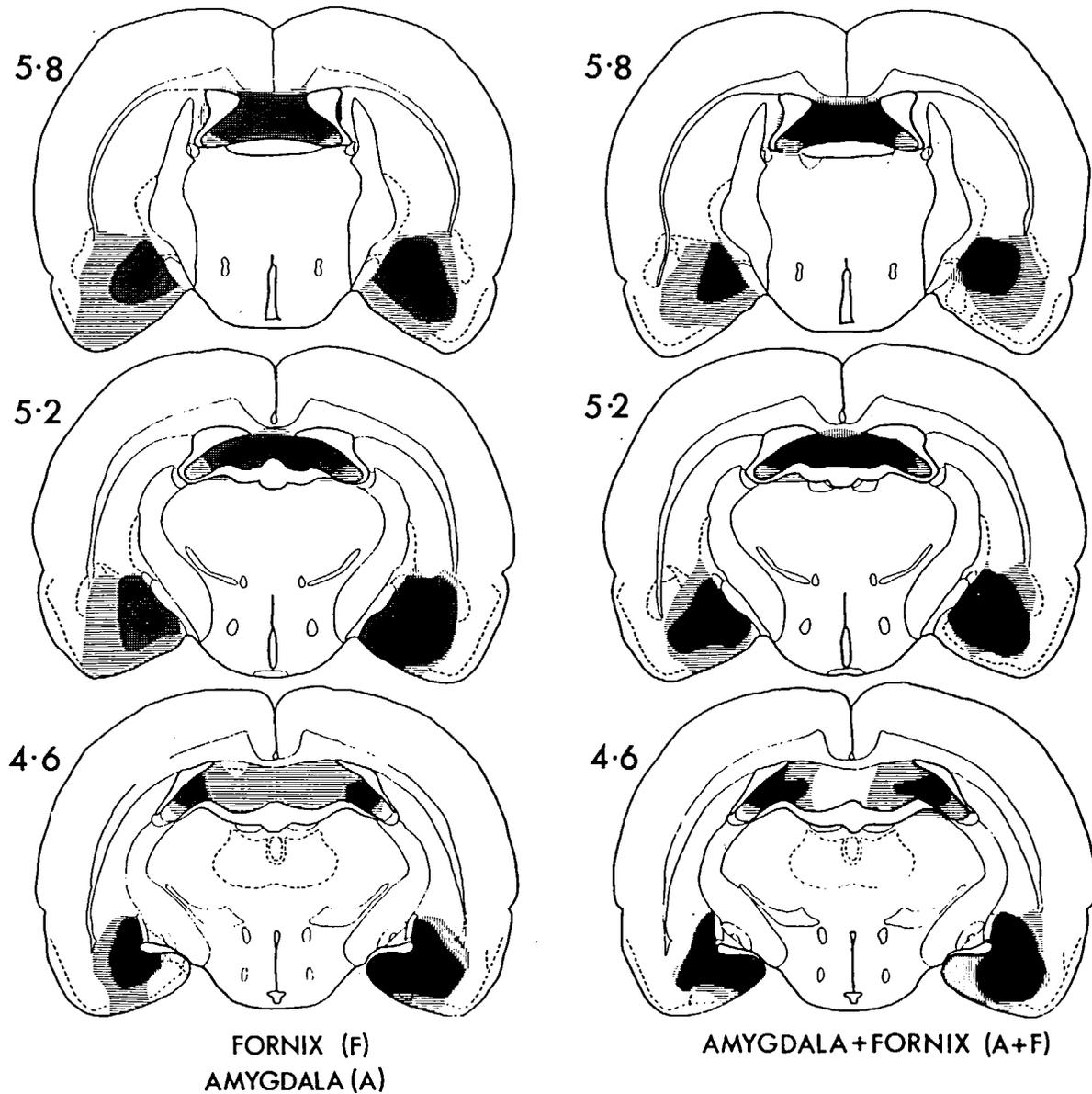


Fig. 1. Diagrammatic reconstruction of the A, F, and A + F lesions. Left: the smallest (vertical lines) and largest (horizontal lines) extent of cellular loss in the amygdala (A group) and fibre loss to the fimbria/fornix (F group). Areas of common damage are in black. Note that the lesions in these two groups are depicted on the same standard coronal sections. Right: the smallest (vertical lines) and largest (horizontal lines) of the fornical lesions in the A + F group. The extent of amygdala cell loss is also depicted for these same two cases. The numbers in parentheses refer to the approximate corresponding AP levels from the stereotaxic atlas of Pellegrino and Cushman²⁸.

gest and the smallest of the lesions in the six remaining F animals are shown in Fig. 1. It can be seen that the surgeries produced very extensive damage to the fimbria/fornix, cutting between 80 and 100% of the fibre tract bilaterally, the lesion often extending into the rostral head of the hippocampus (Fig. 1). In two animals the lesion just touched the most dorsal part of the anterior thalamic nuclei, while in one case there was very slight involvement of the caudate nucleus (Fig. 1). In all cases the corpus callosum was cut but the cingulate regions were spared.

Amygdala plus fornix (A + F). In two of the A + F surgeries approximately 30% of the fimbria/fornix was spared in at least one hemisphere and these animals were excluded from subsequent analyses. In the remaining eight animals the lesions in the fornix and the amygdala were comparable to those in the F and A groups, respectively. In these eight A + F cases between 80% and 100% of the fimbria/fornix was transected, any sparing occurring at the most lateral tips of the fimbria (Fig. 1). Additional damage extended into the dorsal limit of the anterior thalamic nuclei in four cases. The

amygdala surgeries consistently produced extensive cell loss through nearly all of the structure, although in two cases the lateral nucleus was spared unilaterally. In two cases there was also very limited damage to that part of the striatum adjacent to the central nucleus. With a single exception, the sizes of the amygdala lesions in the A + F group were comparable to the larger of the two lesions depicted in Fig. 1.

Sham (ASHAM, FSHAM, A + FSHAM). Six rats received sham surgeries for each of the three lesion groups, making a total of 18 control animals. All surgeries were as intended.

'Spaced' concurrent discrimination

Performance on the 'spaced' problem was assessed by comparing the total number of errors made by each animal before reaching the acquisition criterion score (89%) or the limit of 60 sessions. Non-parametric statistics were used as only 7 of the 18 sham control animals (and 2 of the 6 A animals, none of the 6 F animals, and 1 of the 8 A + F animals) reached the acquisition criterion. As a consequence the error scores were not distributed normally. An initial comparison indicated that the three Sham (ASHAM, FSHAM, A + FSHAM) groups did not differ from one another (Kruskal-Wallis $H = 5.33$) and these scores were therefore combined to form a single SHAM group.

The median error scores (chance = 180) of the four subsequent groups were: SHAM = 145, A = 149.5, F = 175, A + F = 172. Comparisons between these scores provided evidence of a lesion effect ($H = 8.87$, $P < 0.05$), and further tests using the Mann-Whitney statistic indicated that while the A group was unaffected ($U_{18,6} = 62$), both the F ($U_{18,6} = 21$, $P < 0.025$) and the A + F ($U_{18,8} = 41$, $P = 0.05$) groups were impaired relative to the SHAM group. Furthermore, while the F and the A + F groups did not differ from each other, both groups made more errors than the A groups (A vs. F $U_{6,6} = 2.5$, $P < 0.025$; A vs. A + F $U_{6,8} = 9$, $P < 0.05$).

A second measure of performance, the number of sessions required to reach an arbitrary criterion score (14 or more correct trials over 3 consecutive days), was also used to compare the various groups. Analyses using this lower criterion score revealed exactly the same pattern of results as that obtained by comparing total error scores. That is, the three Sham groups did not differ ($H = 0.80$), but comparisons between the SHAM animals and the three experimental groups provided evidence of a lesion effect ($H = 11.8$, $P < 0.01$). Once again, while the A group was unaffected ($U_{18,6} = 44$), both the F group ($U_{18,6} = 13.5$, $P < 0.01$) and the A + F group ($U_{18,8} = 28$, $P < 0.01$) were

impaired relative to the SHAM animals. There was no difference between the two groups with fornical lesions ($U_{8,6} = 22$).

Before concluding that fornical, but not amygdala, damage in rats impairs the ability to learn 'spaced' concurrent discriminations, other possible explanations for the present deficit should be considered. One possibility is that the F and A + F lesions attenuated the strength of the animals' preferences for the stimuli selected on the very first session (i.e. those stimuli that became the positive stimuli). If this had happened, the F and A + F animals might have found it more difficult to learn the subsequent discriminations. Closer examination of initial performance, however, provides no support for this proposal. It was found, for example, that the scores of the various groups during sessions 2–5 were very similar (SHAM 55.8%, A 49.2%, F 57.5%, A + F 50.5%), there being no evidence that the SHAM and the A groups were at an advantage during the very initial stages of the task.

In the light of the considerable evidence that fornical damage disrupts performance on spatial tasks, the total number of side alternations made on successive trials was calculated for the first three sessions. In addition, the proportion of choices between whichever was the preferred side and the other side was calculated for each animal for the same sessions. Like many previous studies it was found that the fornical lesions reduced the number of alternations ($H = 12.9$, $P < 0.05$), with both the F ($U_{18,6} = 17$, $P < 0.01$) and the A + F ($U_{18,8} = 25.5$, $P < 0.01$) groups making significantly fewer side alternations than the SHAM group. In addition, the initial balance of choices between the two food wells revealed evidence of a lesion effect ($H = 10.8$, $P < 0.05$), with the F animals displaying an abnormally strong preference for one well over the other ($U_{18,6} = 10$, $P < 0.01$). The A + F group did not, however, differ from the SHAM controls on this measure ($U_{18,8} = 47.5$). In spite of these differences in spatial responding it was not the case that all of the F and A + F animals developed fixed position habits as a number of animals successfully learnt individual discrimination pairs but performed poorly on the remainder. This is reflected by the lack of an overall group difference when the number of individual pairs 'learnt' (defined as eight or more correct out of the final ten trials) by each animal was compared ($H = 5.84$, mean 1.5).

Lastly, the spaced concurrent discrimination task provides an opportunity to look for evidence of primacy effects in a non-spatial task. That is, if rats are able to take advantage of the greater rehearsal of those items at the beginning of the list they should, all other things

being equal, learn the very first pair of discriminations faster than the others. We therefore examined the last 10 sessions of each of the 18 SHAM animals. There was no evidence, however, that performance on the first pair of stimuli (overall 62.2% correct) was better than that of the five subsequent pairs (65.6%, 63.3%, 53.8%, 74.4% and 67.8% correct, respectively). It should be added that a stronger test for primacy effects would have involved counterbalancing the order of the stimuli between animals.

'Standard' concurrent discriminations

Performance on this task was measured from the individual scores for each of the 36 sessions. These scores were subject to Arcsin transformation to help reduce deviations from normality. An initial analysis of variance of data from just the three sham groups found no evidence of a group difference ($F_{2,15} = 1.23$) or of a session by group interaction ($F_{70,525} = 1.06$). The ASHAM, FSHAM and A + FSHAM groups were therefore combined to form a single SHAM group.

From Fig. 2 it can be seen that, relative to the SHAM group, the F and the A + F groups performed poorly on the 'standard' concurrent discrimination task. Comparisons between the four groups with the factors Lesion and Session indicated that there was both a Lesion effect ($F_{3,34} = 3.48$, $P < 0.05$) and a Lesion \times Session interaction ($F_{105,1190} = 2.37$, $P < 0.001$). As the interaction term reflects differential learning rates it was investigated further by comparing the three experimental groups with the SHAM animals. While there was no interaction between the A and SHAM groups ($F < 1$), both the F groups ($F_{35,770} = 4.10$, $P < 0.001$) and the A + F group

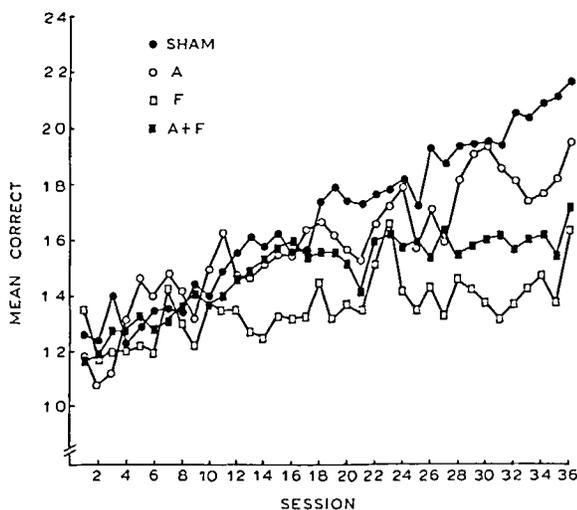


Fig. 2. Mean number of correct responses for the SHAM, A, F, and A + F groups for each session of the 'standard' concurrent discrimination task (six pairs of objects).

($F_{35,840} = 3.29$, $P < 0.001$) revealed highly significant interactions with the SHAM animals, reflecting the much flatter rates of acquisition of the animals with fornical lesions (Fig. 2). Comparisons between the overall scores of the four groups, using the Newman-Keuls test, indicated that only the F group ($F < 0.01$) differed from the SHAM animals on this measure.

It can be seen that, as in the previous task, the two groups with fornical lesions were impaired on a concurrent discrimination task. There was, however, no effect of amygdala damage. Furthermore, the deficit following fornical damage, which was reflected by the flatter acquisition curves, was not accentuated by the amygdala lesions. Indeed, if anything, the impairment was most pronounced in the F group. In order to determine whether the deficit in the animals with fornical lesions could be a direct consequence of a failure to learn the previous 'spaced' discrimination⁴¹, the correlation (Spearman Rank) between total error scores on the 'spaced' and the 'standard' tasks was calculated for the 14 animals with fornical lesions (A + F and F). The lack of correlation ($r = -0.12$) suggested that it was not the experience of the 'spaced' condition that brought about the deficit on the 'standard' condition. Further evidence that failure to master the 'spaced' task did not disrupt performance on the 'standard' task comes from the lack of difference between the performance (the total number of correct trials) of the seven SHAM animals that reached the learning criterion and the eleven SHAM animals that failed ($U = 31$). Lastly, the relative side preferences of the animals in the four groups were compared for each of Sessions 1–3. The lack of overall difference between the groups ($H = 5.30$, 0.93, 2.95, respectively) suggests that the animals with fornix lesions had not simply adopted an extreme position habit that interfered with learning the concurrent discriminations.

'Massed' concurrent discriminations

The mean scores for the 'massed' concurrent discrimination task, in which the animals received 36 trials on each of two discriminations over two sessions within a single day, are shown in Fig. 3. A distinction has been made between those trials in which S+ was the 'preferred' object and S+ was the 'non-preferred' object (i.e. S+ was the stimulus chosen on trial 1 and the stimulus not chosen on trial 2, respectively). As one of the animals from the A group would not complete the required 72 trials within a day its results were discarded, leaving five animals in the A group.

An analysis of variance with the factors Groups, Session, and type of S+ failed to find an overall lesion effect ($F_{3,33} = 2.24$, $P = 0.10$), although there were sig-

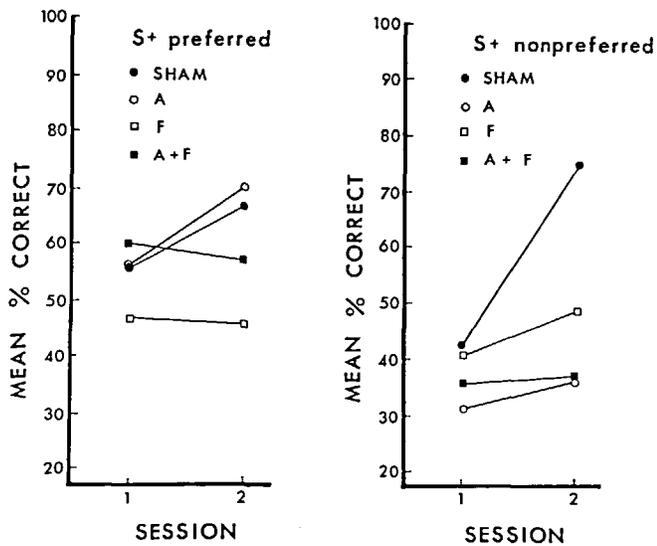


Fig. 3. Mean percent of correct responses for the SHAM, A, F and A + F groups for the two sessions of the 'massed' concurrent discrimination task (two pairs of objects). Left: when the S + is the preferred object, as determined from Trial 1. Right: when the S + is the non-preferred object, as determined from Trial 2. (The responses for Trial 1 and 2 have accordingly been excluded from the measures of overall performance).

nificant interactions between the scores of the groups across the two sessions for both the 'preferred' ($F_{3,33} = 3.35$, $P < 0.05$) and the 'non-preferred' ($F_{3,33} = 5.85$, $P < 0.005$) S + conditions, i.e. the different groups showed different rates of improvement across the two sessions for both the 'preferred' and the 'non-preferred' S +.

Subsequent analyses focussed on the separate scores from the two S + conditions. Particular attention was given to the interaction terms within each condition as these terms reflected rates of acquisition. In contrast, the overall levels of performance were regarded as poorer measures of learning as these were likely to be affected by transfer effects from previous S + or S - stimuli.

Comparisons on the scores from just the 'preferred' condition indicated that while the scores of the A group did not differ from those of the SHAM group, both the F group ($F_{1,22} = 4.30$, $P < 0.05$) and the A + F group ($F_{1,24} = 6.17$, $P < 0.025$) improved at a slower rate across the two sessions (Fig. 3, left). Similar comparisons for the 'non-preferred' S + again indicated that the SHAM group improved at a faster rate than the F ($F_{1,22} = 6.41$, $P < 0.025$) and the A + F ($F_{1,24} = 14.51$, $P = 0.001$) groups. There was also evidence that the A group also improved at a slower rate than the SHAM animals ($F_{1,21} = 5.99$, $P < 0.025$). This apparent difference between the performance of the A group with the 'preferred' (normal) and 'non-preferred' (impaired) stimuli (Fig. 3) was reflected in the interaction between the

SHAM and A animals across the two S + conditions ($F_{1,21} = 9.44$, $P = 0.0058$).

Thus rats with fornical lesions (F or A + F) were impaired on both S + conditions, as reflected by their failure to improve at a normal rate across the two sessions. In contrast, the animals with amygdala lesions were only impaired when they were required to switch their preferences (i.e. select the non-preferred S +).

GENERAL DISCUSSION

The present study found that fornical damage impaired the acquisition of three different concurrent discrimination tasks. These impairments were found irrespective of the number of trials received on each stimulus pair within a session. In contrast, amygdala lesions had no apparent effect on these same tasks. The only exception was in the final 'massed' condition, and then the animals with amygdala lesions were only impaired when the correct stimulus was the object that had originally not been chosen. There was no evidence that the combination of both fornix and amygdala damage produced a greater deficit than that found after fornical damage alone. Lastly, an analysis of the scores from the first concurrent task failed to provide any evidence of a primacy effect on this non-spatial task.

The finding that fornical lesions can impair concurrent discrimination learning provides another example of a deficit following hippocampal system damage attributable to neither working memory³⁰ nor spatial mapping³². It is also unclear as to how the present findings could be accommodated into certain more recent theories of hippocampal function in the rat, e.g. as a temporary memory buffer³⁶ or as a region necessary for configural associations³⁹. There is, however, the possibility that the abnormal spatial behaviour shown by the F and A + F groups could have indirectly impaired the learning of these discrimination problems e.g. through perseverative selection of a particular food well (ref. 15, but see ref. 41). The evidence that such changes led to the 'learning' deficit is, however, unconvincing. For example, although the A + F animals were impaired on the 'spaced' task, the same group did not show an abnormal, initial distribution of choices between the two food wells, i.e. they were regularly selecting both food wells. Furthermore, animals in the A + F and F groups were able to learn individual 'spaced' discriminations, but were unable to master the remainder. In addition, comparisons using the first three sessions of the 'standard' condition showed that the balance of choices between the two food wells by both the F and A + F groups did not differ significantly

from that of the SHAM animals, even though both of these groups were impaired on the task. Finally, on the massed condition animals in both the F and A + F groups were consistently performing at below chance for the 'non-preferred' S + (Fig. 3), highlighting their ability to select both sides of the apparatus.

Other relevant evidence comes from the frequent finding that hippocampal damage does not disrupt the acquisition of simple, simultaneous discriminations^{14,20,27,40,41} unless the training is preceded by a conflicting task⁴¹. Even stronger evidence comes from a similar study⁴⁰ showing that neither fornix, hippocampus, or amygdala plus hippocampus damage disrupts the acquisition of a single object discrimination by rats, even though all three lesions disrupt the acquisition of a concurrent task similar to the 'standard' condition⁴⁰. Further evidence that the effects of fornical damage on spatial choice need not indirectly impair an object discrimination task comes from the discovery that comparable fornical lesions do not disrupt the acquisition of a delayed nonmatching-to-sample task in a Y-shaped maze⁴. Indeed, it could be argued that rats with fornical damage might be able to learn some non-spatial tasks faster than normal animals as they are less likely to rely on irrelevant spatial cues⁶.

Deficits in the learning of discriminations following hippocampal system damage have been related to a number of factors including low or differential saliency between the stimuli^{8,35}, or experience on an interfering problem⁴¹. While the present study does not address the first of these factors, there is clear evidence that the present learning deficit was not simply a consequence of interference from preceding problems (see also ref. 40). The impairment found on the very first concurrent task ('spaced') i.e. prior to any conflicting training, runs counter to this explanation. Other inconsistent evidence comes from the failure of the F and A + F animals to improve when the S + on the 'massed' task was the 'preferred' object. From Fig. 3 (left) it would appear that the A + F animals started with a clear preference for the S + but were still unable to improve with further practice.

The present results can be seen as consistent with studies using monkeys which have indicated that hippocampal damage disrupts the acquisition of concurrent discriminations similar to the 'standard' task used in the present study^{21,28,42}. But, while both sets of studies point to the involvement of the hippocampal formation in such learning, an obvious difference is that fornix transection does not appear to disrupt concurrent learning in the monkey^{28,43}. This finding, along with other similar results from studies of monkeys⁴³, suggests that there may be a different balance in the

functional importance of certain key hippocampal connections across these species. In particular, it may be that in rats the direct diencephalic connections via the fornix are important for a wide range of hippocampal functions, but that in macaque monkeys some of these same functions are subserved by the extensive temporal interconnections of the hippocampal formation, connections that provide an indirect route to the diencephalon. As a consequence there may be a change in the relative contribution of the fornix across species.

The issue of species differences is also raised by the results of the 'spaced' concurrent task. It was found that lesions to the fornix or to the fornix plus amygdala impaired acquisition equally on this type of concurrent discrimination learning. These results differ from those obtained from macaque monkeys where performance on 'spaced' concurrent learning is seemingly unaffected by combined amygdectomy and hippocampectomy²². It is argued that this sparing is a consequence of non-limbic learning systems (e.g. cortico-striatal) that are of particular importance when individual trials are spaced, and training is lengthy^{25,34}. While this apparent species difference might reflect a reliance on slightly different sensory cues, it should be noted that an attempt to replicate the finding from monkeys with human amnesic subjects also found no such sparing³⁸ i.e. both the human amnesic subjects and the rats in the present study were impaired on the acquisition of 'spaced' concurrent discriminations.

◀ For the majority of the concurrent discrimination tasks the neurotoxic amygdala lesions had no apparent effect. The finding that both the 'spaced' and the 'standard' conditions were unaffected runs counter to the most general version of the proposal that the amygdala is required for the normal acquisition of stimulus-reward associations^{16,24}. A more refined version of this proposal is that the amygdala is only necessary when the acquisition of stimulus-reward associations is rapid^{1,25} and that other neural systems are employed when training is lengthy. This proposal was tested in the 'massed' condition where the amygdala lesions appeared to have a differential effect: they did not alter learning when the S + was the preferred stimulus, but when the S + was the non-preferred stimulus the animals with amygdala lesions were impaired. This result closely resembles the finding of Schwartzbaum and Poulos³⁷ who reported that the learning-set deficit following amygdectomy in monkeys is most evident when the animals have to switch preferences. These results, taken together with those from discrimination reversals^{2,16,17,37}, might indicate a special involvement of the amygdala when stimulus selection is repeatedly incorrect and hence linked with affective reactions. Just such

a proposal has recently been made^{9,18}. This cannot, however, be a complete description as deficits following amygdectomy are also found on learning-set tasks in which there should be no obvious heightened affective reactions^{1,2}.

One of the prime purposes of the present study was to determine if the effects of amygdala damage can accentuate those of fornical damage. This arose from the proposal, based on studies of recognition memory in monkeys^{7,23}, that combined damage to the amygdala and hippocampus was responsible for the full anterograde amnesic syndrome. This proposal has received some support from previous studies of rats which indicated that a combination of limbic lesions may enhance certain recognition deficits^{1,3}. There was, however, no evidence from the present study for an analogous combinatory effect on concurrent discrimination learning (see also 39). The same conclusion comes from a recent study into the effects of limbic lesions in rats on the acquisition of a five-pair concurrent discrimination task⁴⁰. It was found that lesions in the hippocampus, fornix, or hippocampus plus amygdala all produced impairments on the concurrent discrimination task and that these impairments did not differ from one another⁴⁰. It can be seen that, taken together, these findings appear more consistent with recent data indicating that a major part of the 'amygdala' lesion effect in monkeys may be a consequence of inadvertent damage to the perirhinal and entorhinal cortices^{29,44}. If this is proven to be the case then one might not expect neurotoxic amygdala lesions to potentiate the effects of hippocampal system damage on concurrent discrimination learning tasks.

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