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THE EFFECTS OF HIPPOCAMPAL LESIONS UPON SPATIAL AND NON-SPATIAL TESTS OF WORKING MEMORY

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A series of experiments examined the proposal that the primary effect of hippocampal damage in rats is to disrupt working memory. Although extensive hippocampal lesions produced a severe impairment in forced-choice alternation – a test of spatial working memory – the same lesions did not impair the acquisition of a non-spatial test of working memory – delayed non-matching-to-sample. This test of object recognition required the rats to select that arm in a Y-maze which contained unfamiliar stimuli. Rats with hippocampal lesions were able to learn and perform this task at normal rates, even with retention delays of as long as 60 s. Two additional experiments helped confirm that the animals had indeed learnt a non-spatial test of working memory. The final experiment examined whether hippocampal lesions resulted in an increased sensitivity to proactive interference. It was found that repetition of test stimuli within a session, which increased interference, did attenuate recognition performance but there was no evidence that the animals with hippocampal lesions were differentially affected.

INTRODUCTION

Considerable interest has been aroused by the proposal that hippocampal damage selectively disrupts working memory in rats[19]. This proposal argues that the hippocampus and its primary afferent and efferent connections are necessary for learning flexible stimulus–response associations such as information which is useful for only one trial of an experiment but is irrelevant, or even misleading, on subsequent trials (working memory). In contrast, the learning of constant response associations (reference memory) does not require the hippocampus. Thus a hippocampal lesion should not affect learning always to turn left in a T-maze (reference memory) but will disrupt discrete-trial alternation (working memory) which requires remembrance of the previous trial[19,26]. The emphasis of this hypothesis on the mnemonic functions of the rat hippocampus has proved particularly attractive in the light of the human amnesic and monkey experimental literature which has repeatedly shown that the hippocampal formation has a key role in memory[5,29].

Much of the evidence for the importance of the hippocampus in working memory has come from studies of spatial learning by rats[19]. There is little doubt that hippocampal system lesions severely disrupt tasks such as discrete-trial spatial alternation, complex maze learning and the radial arm maze devised by Olton and Samuelson[21]. It has been strongly argued, however, that the observed deficits are a consequence of the spatial nature of the tasks rather than the requirement for working memory[16,18]. One direct way of distinguishing between these alternatives would be to examine

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the effects of hippocampal damage on a non-spatial test of working memory.

Olton and Feustle[20] reported that fornix transection impaired the ability of rats to remember the appearances of those arms in a maze which had already been entered during a session. This working-memory task could not be solved by using spatial memory as the relative positions of the arms were changed after each choice-trial. In contrast, other experiments which tax the remembrance of cues indicate that hippocampal lesions can leave working memory intact[11,14]. These apparently conflicting results have lead us to re-examine this critical aspect of the working-memory hypothesis.

EXPERIMENT 1

The experimental task was deliberately modelled on tests of object recognition (delayed non-matching-to-sample) which have proved sensitive to limbic and diencephalic damage in monkeys[2,15]. The rats were tested in a Y-maze in which the start box and the two goal boxes were removeable. On each trial the start box matched one of the goal boxes and differed from the other. The rats were rewarded for selecting the goal box that differed from the start box. This task taxed working memory, as both the start and goal boxes changed after every trial (Fig. 1) and although the task might be regarded as a simultaneous oddity problem, previous observations have shown that rats rarely attempt to make a direct comparison of all 3 boxes.

Method

Subjects. The subjects were 18 naive, male pigmented rats of the DA strain (Bantin and Kingman, Hull, U.K.) which were caged individually. The animals were fed ca. 15 g of laboratory diet daily so that their weights did not drop below 85% of normal body weight. The rats, whose inital ages ranged from 7–9 weeks, were allocated randomly to 3 surgical groups.

Six additional male DA rats who had learnt the recognition task prior to surgery were included in Experiment 1. These rats, which were ca. 8 months old at the time of surgery, were housed and fed in the same manner as the naive animals.

Apparatus

Each arm of the aluminium Y-maze was 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 constituting each pair was made as similar as possible but each pair was distinct from every other pair. To this end the walls and floors of the boxes were painted in different colours and patterns, and the floors were lined with a variety of materials such as sandpaper, wooden strips, metal, perspex and cloth. 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 (Fig. 1). 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. Further details of the apparatus have been published[1].

The same apparatus was used for the animals who had learnt the non-matching task prior to surgery with one minor difference: only 40 pairs of start/goal boxes were used as only this number of boxes had been used during their preoperative training.

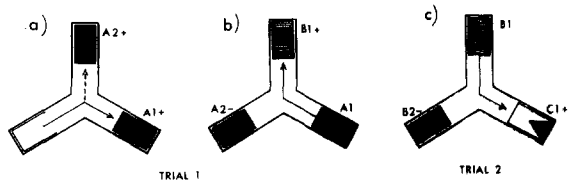

Fig. 1. Diagrammatic representation of the non-matching-to-sample procedure in which the animal was rewarded for choosing the novel goal box. Arrows indicate direction of correct responses.

Surgical

All the rats were anaesthetized by intraperitoneal injection of 3 ml/kg of chloral hydrate–pentobarbitol mixture (containing 42 mg/ml chloral hydrate and 9.7 mg/ml Nembutal). They were then placed in a stereotaxic headholder and the scalp was cut and retracted to expose the skull. A dental drill was used to make an opening exposing the dura overlying the dorsal hippocampus. The rats receiving hippocampal lesions (HPC, n = 12), were transferred to a specially designed headholder[25] and had the dura sectioned and the necessary neocortex removed to allow the hippocampus to be aspirated. All aspirations were carried out under visual control, using a Wild M650 operating microscope. The rats receiving cortical control lesions (CORT, n = 8) had an equivalent amount of neocortex and cortex callosum aspirated, exposing the alveus of the dorsal hippocampus. Both groups had the wound packed with Sterispon gelatine foam soaked in physiological saline. The sham-operated rats (SHAM, n = 4) had the dura left intact. Sulphanilamide powder was applied in all the rats and the skin was then sutured.

Histological

At the end of the study the CORT and HPC rats were perfused intracardially with 5% formol 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 cresyl violet.

The HPC surgeries produced extensive bilateral damage to the hippocampus and in all cases a large majority of the structure was removed (Fig. 2). In addition, the fimbria and fornix were either completely sectioned or displayed dense gliosis. Only the most caudal and the most ventral portions of the hippocampus were spared and in some cases even these regions were damaged. In all but one case there were small infarcts in the thalamus. These infarcts, which were usually bilateral, were concentrated in the lateral dorsal nucleus and, to a lesser extent, in the anterior nuclei.

Damage to the cortex appeared comparable in the HPC and CORT animals and in all of the CORT animals the corpus callosum was completely sectioned. In only one CORT case was there evidence of minor, unilateral damage to the alveus, the most superficial portion of the hippocampus. Two of the CORT cases had small infarcts in the lateral dorsal nucleus of the thalamus.

Behavioural

Following a period of pretraining, which involved handling the rats daily and training them to run in the Y-maze for food pellets, the experiment began. To start each test-session the rat was placed in an arm with a featureless start 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, Fig. 1a). The rat was deemed to have made a choice when all 4 paws had entered an arm, whereupon the guillotine 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 (Fig. 1b). 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 (Fig. 1c). 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 fifth 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. During these correction trials the goal boxes were rearranged so that entering the positive box required the same body turn as in the test trial. These correction trials were necessary as they

Fig. 2. Reconstruction (left) of the smallest (black) and largest (diagonal lines) HPC lesion on 3 coronal sections. The numbers in parenthesis refer to the corresponding plates in the atlas of Pellegrino and Cushman[23]. Alongside (right) are two photomicrographs showing the appearance of the HPC and CORT lesions (Nissl stain).

ensured that the animal entered the goal box which was to become the next start box.

When a rat reached the criterion score of 40 or more correct responses in 5 consecutive days (80%), the second phase of the experiment began. The rat's ability to remember the test boxes was now assessed for 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 days at each of the 3 conditions in a counterbalanced order. All behavioural testing was blind.

Statistics

The results from all the experiments reported here were analysed in the same way: choice accuracy for each subject was expressed as a percentage of the maximum score possible, and this datum was submitted to an analysis of variance using a design in which the two control groups were represented by a factor of Control-Type nested within the factor Lesion. Where the two control groups did not differ significantly from one another, we report the results in terms of the Lesion factor; where the two control groups did differ significantly, we report both the results of Lesion and Control-Type. These analyses were carried out with no further transformation of the data, using the GENSTAT package implemented on an ICL 2900 computer. Trend analysis, using the method of orthogonal components, were included in the analysis where appropriate, and a split layer design was used to analyse experiments in which repeated measures had been taken.

In Experiment 1 the comparison of the postoperative acquisition at different interresponse delays was analysed using the factors Lesion, Control-Type and Delay. Comparisons of pre- and postoperative performance was analysed using the factors Lesion, Operation (with two levels: Preoperative and Postoperative) and Delay.

Results

Fig. 3 (left) shows the number of trials preceding the 80% criterion learning score. There were no differences between the 3 groups ($F < 1$) with the SHAM controls requiring a mean of 190 trials, the CORT controls 232 trials, and the HPC animals 235 trials. The animals were further tested by comparing daily performance over 50 trials at each of 3 retention delays 0 s (training condition), 20 s and 60 s (Fig. 3, right). Although delay impaired performance ($F = 13.8$, df = 2,28, $P < 0.01$), and it was clear that the decrement was only significant at the 60 s delay (minimum $t = 4.4$, df = 28, $P < 0.01$), there were no differences between the experimental groups ($F < 1$). It should also be noted that the 3 groups were still performing well above chance after the longest retention delay (Fig. 3).

The same pattern of results was observed from the 6 animals who had learnt the recognition task before surgery and had been retested following either hippocampal or cortical control lesions (Fig. 4). Unfortunately one animal died following surgery leaving just two control animals. Nevertheless, there was no evidence that the intervening surgeries differentially affected the ability to regain the criterion performance level as the two control animals required 0 and 50 trials and the 3 HPC animals required 0, 60 and 120 trials. Comparisons between the preoperative and postoperative scores on the 3 delays (Fig. 4) revealed no lesion effect ($F < 1$) although, once again, the effect of delay was significant ($F = 12.9$, df = 2,12, $P < 0.01$) with performance being worst on the 60 s delay condition (minimum $t = 4.4$, df = 12, $P < 0.01$).

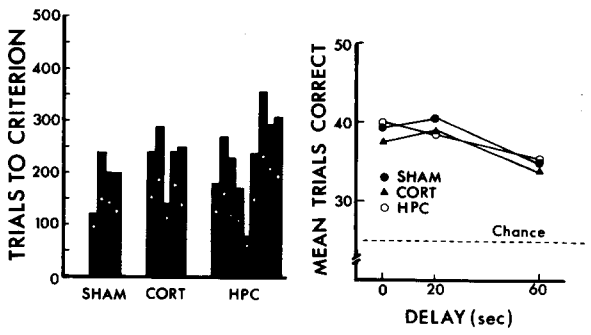

Fig. 3. Non-matching-to-sample. Left: bars indicate trials to 80% criterion level (excluding criterion trials), white circles represent error scores. Right: performance with increasing retention delays between stimulus presentation and choice test.

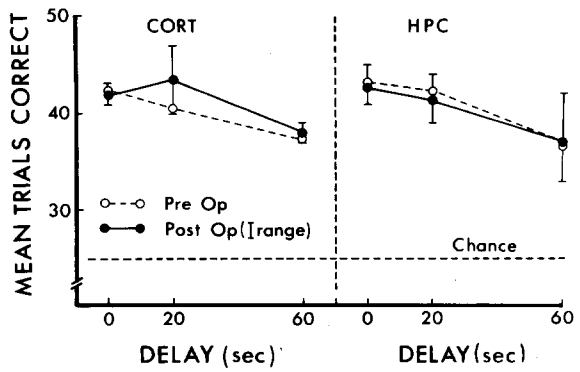

Fig. 4. Non-matching-to-sample. Comparison between preoperative and postoperative retention performance for 2 CORT and 3 HPC animals.

Discussion

The results show that the extensive hippocampal lesions did not alter the ability to learn the object recognition task or to perform it over delays of up to 60 s. This appeared to be true whether the hippocampal lesions were given before or after acquisition of the recognition task. Thus in contrast to the findings of Olton and Feustle[20] there was no support for the proposition that damage to the hippocampal system will disrupt both spatial and non-spatial tests of working memory.

One possible explanation for this discrepancy is that there was some reference memory solution available in the current task which accounts for its apparent insensitivity to hippocampectomy. Thus if the rats had discovered some constant, unintended cue, e.g. an auditory cue from changing the boxes which predicted the correct arm, our results would still be compatible with the working memory hypothesis. This possibility was examined in Experiment 2.

EXPERIMENT 2

This control experiment used exactly the same testing procedure as Experiment 1, except that only two pairs of start/goal boxes were used throughout (object alternation). This modification meant that, except for the first trial of each day, the two goal boxes were closely matched in their familiarity and there was a considerable increase in proactive interference. If there was an unintended reference memory solution, which did not depend on the capacity to remember the identity of the box most recently visited, this procedural change should not affect task performance.

Subjects

For this and all subsequent experiments the total number of subjects was 16 (HPC n = 8; SHAM n = 4; CORT n = 4). These subjects consisted of all of the animals from Experiment 1 which had received surgery before learning the recognition task, with the exception of one CORT and one HPC animals which had died during the intervening period.

Method

The apparatus and testing procedure were identical to those used in Experiment 1, with one important exception: just two pairs of dissimilar boxes were used during each session. This change meant that the sequence of rewards became A + vs B − (trial 1), A − vs B + (trial 2), B − vs A + (trial 3). Each animal received 50 trials over 5 days and correction trials were run as in Experiment 1. The same pair of boxes were used on each day. Testing began one week after the last animal had finished Experiment 1.

Results

Fig. 5 compares the percentage of correct responses on trial 1 and trials 2–10. As predicted, the animals were able to respond significantly above chance on trial 1 (mean 77.5%, $t = 8.88$, df = 15, $P < 0.01$). In contrast, the animals could not solve the subsequent trials (2–10), during which both sets of boxes had become familiar. While the scores of the HPC animals did not differ from chance (Fig. 5), those of the combined CORT and SHAM groups were significantly below chance ($t = 4.22$, df = 7, $P < 0.01$). An analysis of choice accuracy as a function of Trials (with 10 levels), Lesion and Control-Type confirmed

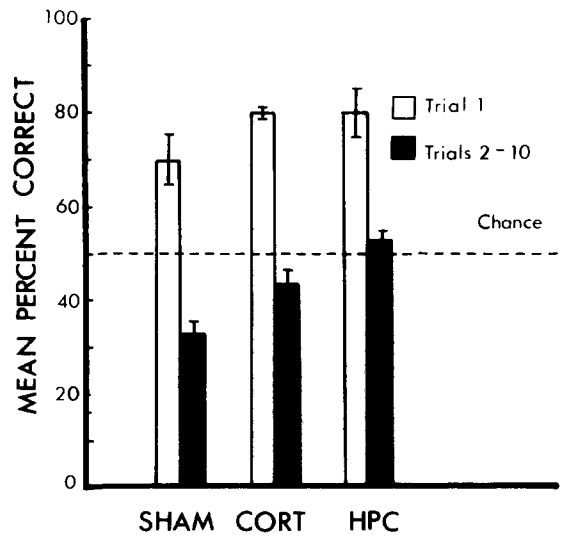

Fig. 5. Object alternation. Percent correct responses on trial 1 and trials 2–10 averaged over the 5 test sessions. Vertical bars show standard errors.

that performance differed with respect to trial position ($F = 3.5$, df = 9, 117, $P < 0.01$) and that there was a lesion effect ($F = 23.3$, df = 1,13, $P < 0.01$). Inspection of the data revealed that the scores of the two control groups were significantly below chance on those trials in which correct box was in the arm the animal had just run from ($t = 3.3$, df = 7, $P < 0.02$). When the correct box was in the other arm, the performance of the control groups did not differ from chance. This reluctance to return to the same arm and box that had just been visited accounted for the differences with the HPC animals.

Discussion

There was no evidence that any of the animals had discovered an alternative stratagem for solving the non-matching task as all animals failed a control task which differed from Experiment 1 in only one respect; the same two pairs of start and goal boxes were used for every trial. This difference ensured that, with the exception of trial 1, the task could not be solved by choosing the highly unfamiliar and avoiding the recently familiar stimulus. Furthermore, the design of Experiment 1 ensured that the rats could not use their own odour trails in order to avoid the familiar box. This possibility was excluded as each discrimination was between two boxes the animal had not entered during that session, even though one appeared familiar. Thus there was no evidence that the HPC animals had employed any unintended reference memory solution to Experiment 1. Furthermore, the results also show that the animals did not treat Experiment 1 as a simultaneous oddity problem but, rather, novelty provided the critical cues for accurate performance.

The next step was to demonstrate whether the hippocampal lesions were sufficient to disrupt spatial working memory. The animals were therefore trained on a rewarded alternation task run as a test of working memory in an elevated T-maze. The experimental procedure was modelled on that described by Rawlins and Olton[26], in which the animal is forced to enter one arm of the T-maze and then on the next run must enter the other arm to receive a food reward. As the rat has to remember which of the two arms it had entered on the previous 'information' run, this is a test of working memory.

EXPERIMENT 3

Method

Apparatus. 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. Testing was carried out in the same room as the previous two experiments.

Behavioural

Testing began 3 weeks after completion of Experiment 2. Each animal received 3 or 4 days of pretraining and by the final day the animals had learnt to run down the stem of the maze to find food pellets at the choice point and in both the food-wells.

At the start of each trial, which consisted of two stages, 3 food pellets were placed in each food-well and a wooden block was placed in one arm close to the choice point. The rat was then placed in the start box and the guillotine door raised. 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 was removed. The guillotine door was then raised and the rat was now free to enter either arm. 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 was 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. In this manner each animal was

rewarded for selecting the arm it had not entered on the forced 'information run'.

The rats were tested in groups of 4 with each rat having one trial in turn. As a consequence the intertrial interval ranged from 3–5 min. The animals received 6 trials each day, each consisting of two runs through the maze. Every rat received 3 forced right and 3 forced left trials every day, although consecutive rats were run on different schedules. The animals were tested for a total of 12 days (72 trials).

Results

Fig. 6 shows the mean performances of the 3 groups over successive blocks of 3 test sessions (18 trials) and illustrates the striking impairment shown by the HPC group. The percent choice accuracy for each rat for each day of testing was calculated. These data were submitted to an analysis using the factors Lesion, Control-Type and Days (with 12 levels). There was a massive effect of Lesion (F = 151.3, df = 1,13, $P < 0.01$) which reflected the poor performance of the HPC animals. While the mean percent correct over all 12 days was 90.1% for the SHAM group and 91.7% for the CORT group, the 8 HPC animals averaged only 53.8% correct, a score which did not differ significantly from chance (t = 1.39). It should be noted that the poor performance of the HPC animals did not correlate with the varying degree of thalamic damage which most of these animals suffered.

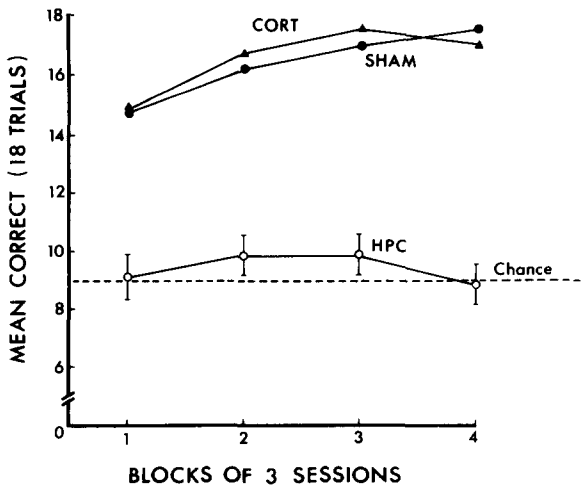

Fig. 6. Forced-choice spatial alternation. Mean performance over successive blocks of 3 test sessions. Vertical bars show standard error of HPC scores.

Discussion

In contrast to the CORT- and SHAM-groups none of the animals with HPC lesions were able to master the spatial alternation task (Fig. 6). In fact, the overall scores of the HPC group did not differ from chance. This result is entirely consistent with previous studies showing that damage to the hippocampal system severely disrupts tests of spatial working memory[19,22,26]. This result also accounts for the differences in the error scores in Experiment 2 in which only the CORT- and SHAM-groups appeared to use spatial information, a strategy which led to an increased number of errors.

The present study has revealed a striking dissociation between the normal behaviour of the HPC animals on a non-spatial test of working memory (Experiment 1) and a spatial test of working memory (Experiment 3). It is unlikely that this dissociation reflects the comparative difficulties of learning these two tasks as the HPC animals were impaired on what would seem to be the easier of the two tasks, the discrete-trial spatial alternation. It would therefore seem that the two tasks differ in some critical manner which distinguishes the effects of hippocampal damage.

EXPERIMENT 4

Although the testing procedure in Experiment 1 precluded the use of odour trails, it is nevertheless possible that the rats learnt an olfactory recognition task. That is, the rats may have learnt to select the goal box which smelt dissimilar to the start box. Although the goal boxes were made from inert material and there was no intention to provide olfactory cues, it is not unreasonable to assume that an animal which is so sensitive to olfactory information might have utilised such cues. In this next experiment we measured the effect of a strong-smelling odour mask upon performance of the object recognition task.

Method

Subjects and apparatus. The subjects were the same as those in the previous two experiments and the apparatus was that used in Experiment 1.

Behavioural procedure

Testing began immediately after completion of Experiment 3. The procedure for the first 5 days was identical to the training condition of Experiment 1. Thus the animals received 10 trials a day on the standard object recognition task (non-matching-to-sample with trial unique stimuli) with 0 s delays. Following this training period the animals received a further 10 sessions in which the centre of the Y-maze, the choice point, was sprayed with an aerosol air freshener ('Haze', Reckitt and Colman Ltd., Hull) which has both a strong and persistent odour. These sessions allowed the animals to become accustomed to the odour and so reduce possible 'generalization decrement' effects[9,12]. That is, the magnitude of the difference between the training and test conditions was reduced so that this difference alone could not account for any change in performance, i.e. 'generalisation decrement'. The odour was reapplied after every fourth test animal and the order of testing varied between days. The animals then received 5 final sessions in which both the maze and all of the start/goal boxes were sprayed with the same persistent odour.

Results

Fig. 7 shows the performance of the 3 experimental groups over the 4 blocks of 50 trials. In addition, we have included the percentage correct on the 48 normal trials, at the 0 s training condition, which the animals performed immediately afterwards as part of Experiment 5. An analysis of variance confirmed that performance improved over the blocks of trials ($F = 15.5$, df = 4, 52, $P < 0.01$) although there was no lesion effect ($F < 1$). This gradual improvement was largely a consequence of the poor scores on the first 50 trials and presumably reflects the interval of ca. 10 weeks between Experiments 1 and 4 and the disruptive effects of Experiments 2 and 3.

None of the critical comparisons indicated that the odour mask disrupted performance. Comparisons between the first and second blocks of 50 trials (the first use of the odour mask), between the fourth block of 50 trials (when both the boxes and the maze were sprayed) and the previous 50 trials, and between the final 50 trials and the scores from Experiment 5 all failed to reach significance ($t < 1$, $t = 1.2$, $t = 1.6$, respectively, df = 52).

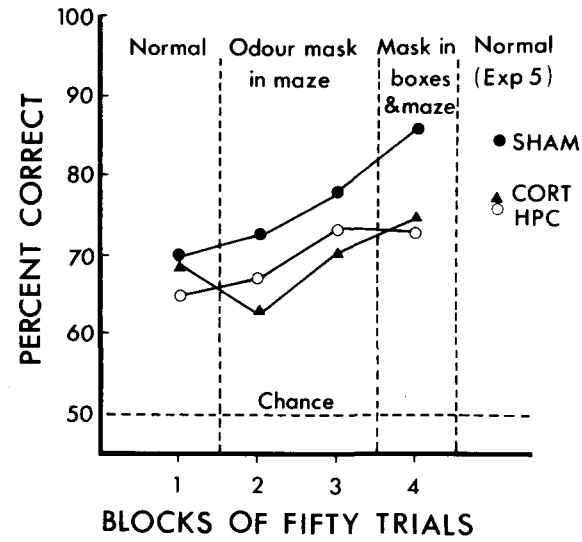

Fig. 7. Olfactory control task. Mean performance on non-matching-to-sample with olfactory mask. Results from Experiment 5 depict the mean percentage correct for the 4 normal test sessions.

Discussion

The application of a strong odour to both the test boxes and the maze had no apparent effect upon the recognition task. This result indicates that the animals did not rely on olfactory cues. This conclusion is consistent with the fact that some of the boxes were composed of the same materials which were arranged in different configurations. These results, combined with those from Experiments 1 and 2, show that the animals had learnt a test of either visual or tactile non-matching-to-sample. Observations of animals performing the task suggested that the visual cues were of importance as 'vicarious trial and error behaviour' was not consistently observed. Nevertheless, it is quite plausible to assume that the animals may sometimes have used tactile or vibrissal cues.

EXPERIMENT 5

One striking difference between the object recognition task (Experiment 1) and the spatial alternation task (Experiment 3) is the degree of proactive interference between the test trials. In the spatial alternation task intertrial interference is maximized as the animals are repeatedly exposed to the same two goal boxes. Thus the ability to make the correct choice depends on a recency judgement between two relatively familiar goal arms. In contrast, the object recognition task minimized intertrial interference by the use of multiple start/goal boxes which were changed after every trial and only repeated every fifth day. As a consequence, it is unlikely that the animals were confused by previous trials involving the same objects. This difference in intertrial interference may be critical as it has been argued that the main effect of hippocampal lesions in rodents and primates is to increase sensitivity to interference and hence bring about faster forgetting[8,30,31].

In order to test this possibility the recognition task was repeated using a restricted set of start/goal boxes. As each box recurred several times within a single session the intrasession interference was increased. If hippocampal lesions do increase sensitivity to interference this manipulation should differentially affect the experimental groups.

Method

The apparatus and subjects were the same as those used in the previous experiment and testing began immediately after its completion. The testing procedure was identical to that used in Experiments 1 and 4, in that the animals were tested on the non-matching-to-sample paradigm with 0 s retention delays.

In the present experiment the animals received 12 trials a day and were tested for 8 sessions. Half of these sessions were normal with 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 objects A,B,C,D which occurred in 3 different consecutive sequences. As in the previous experiments the animal was rewarded for entering the box which was dissimilar to the start box. An example of the sequence of test boxes is as follows; A+ Z−; B+ A−; C+ B−; D+ C−; A+ D−; C+ A−; D+ C−; B+ D−; A+ B−; C+ A−; D+ C−; B+ D−. This procedure meant that on trials 1–4 each positive box was novel, on trials 5–8 each box had been seen once before during that session, and on trials 9–12 each box had been seen twice before. A different set of 4 boxes was used on each of the 4 test days.

Results

Fig. 8 (left) shows the mean performance of the experimental animals on the 4 normal sessions and the 4 with repeated stimuli. Only the last 8 trials of each session were considered, as it is only on these trials that repetitions of the test stimuli occurred. An analysis of variance showed that while the use of repeated stimuli produced a reliable decline in the performance of the recognition task ($F = 11.8$, df = 1,13, $P < 0.01$), there was no effect of lesion ($F = 1.0$) and no interaction between lesion type and trial type ($F < 1$).

An alternative way of presenting the data is shown in Fig. 8 (right) in which recognition performance is plotted against the number of intervening test boxes between the reoccurrence of the same goal box. These numbers range from 1 to 5, with each interval occurring at least 3 times within the 32 trials. The results for 10+ stimuli come from the last 8 trials of the 4 intervening normal sessions. A comparison of the percent choice ac-

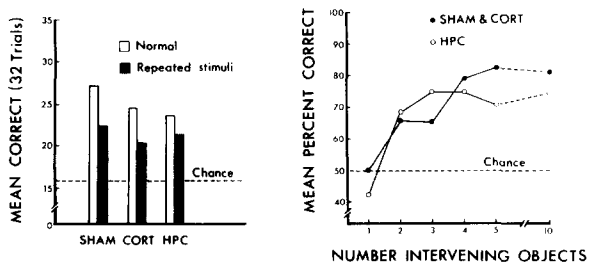

Fig. 8. Proactive interference. Left: mean performance over 32 trials in which the positive stimulus had already been presented during that session, and 32 normal non-matching-to-sample trials. Right: performance plotted against the number of intervening stimuli between the now positive 'unfamiliar' stimulus and its previous presentation.

curacy for each rat for each number of intervening items revealed no effect of lesion ($F < 1$) but a significant effect of intervening items ($F = 3.8$, df = 5,65, $P < 0.01$). As expected the animals performed worst when the number of intervening boxes was low, forcing the animal to select a recently visited box as the 'novel' alternative and an interval of just one stimulus produced the poorest performance (minimum $t = 2.3$, df = 65, $P < 0.025$). The animals' scores improved with an increasing number of intervening stimuli so that after only 3 boxes the scores did not differ significantly from those with 10 or more intervening stimuli.

Discussion

This experiment directly tested the proposal that the deficit shown by the HPC group reflects an increased sensitivity to proactive interference[8,30,31]. It was found that hippocampal damage had no effect both on the original nonmatching task and on a variant in which test stimuli were repeated within a session. This finding appears to rule out the suggestion that the poor performance of the HPC animals on the spatial alternation task was due to the high levels of intertrial interference that might be produced by the experimental design.

Additional evidence that the HPC animals were not abnormally sensitive to interference is found in the first object recognition experiment (Experiment 1). It is quite plausible that a build-up of proactive interference should occur within each object recognition session, as some of the boxes contain similar objects and have similar floors. As a consequence, one might expect the HPC animals to be slightly impaired on the last few trials within each session. A comparison, however, between the scores for the first 5 trials and the last 5 trials of each of the 15 performance test sessions in Experiment 1 revealed no evidence that the HPC animals differed from the CORT and SHAM animals ($F < 1$).

A similar conclusion was derived from the outcome of the first daily trial of the spatial alternation task (Experiment 3). On this trial the animals should suffer the least intertrial interference as they have not entered either test arm for 24 h. Thus one might expect the HPC animals to perform far better on trial 1 than on trial 6. An examination of the scores for the first test trial within each session reveals, however, that the HPC animals performed no better than chance. Similar findings have been reported for rats with restricted septal lesions[26].

Lastly, it should be noted that hippocampal system lesions impair spatial memory tasks, such as the radial arm maze, when the number of places to be remembered is much greater than two and when each place is only visited once during each session[19]. In conclusion, there appears to be no support for the notion that hippocampal damage produces greater sensitivity to proactive interference.

GENERAL DISCUSSION

One of the major criticisms of the proposal that the hippocampus is necessary for normal working memory functions is that the relevant experiments have relied on tests of spatial memory. In the present study we examined the performance of rats with hippocampal lesions on two tests of working memory, one spatial the other non-spatial.

The first experiment found no evidence that extensive hippocampal lesions disrupted either the acquisition or subsequent performance of a stimulus recognition task. Additional experiments helped confirm that the animals had indeed learnt a non-spatial working memory task and indicated that the experiment taxed visual recognition memory. The normal performance of the animals with hippocampal lesions persisted even when a limited set of test stimuli were repeated within a session; the resulting decrease in choice accuracy was equivalent across our 3 treatment groups. In striking contrast these same rats displayed a severe impairment when performing a spatial working memory task, forced-choice alternation. This dissociation, which is similar to that reported by Jarrard[11], is incompatible with the present working memory hypothesis[19].

Several other studies have examined one-trial recognition in rats with damage to the hippocampal system. Gaffan[6] reported that fornix tran-

section impaired the ability of rats to recognise the altered arm in a T-maze while Lukaszewska and her co-workers[14] reported similar impairments in rats with very limited hippocampal lesions. It should be noted, however, that the hippocampal deficit disappeared when the rats were allowed to enter and explore the distinctive arms in the T-maze, prior to the choice-trial[14]. Furthermore, a modified radial-arm maze task devised by Jarrard[11], in which the rats had to identify the distinctive arms they had already entered on that session, also indicated that hippocampal lesions could leave working memory intact. None of these tasks, however, can be regarded as pure tests of non-spatial memory, as remembrance of the location of the distinctive arms would help performance.

A purer test of object recognition was employed by Olton and Feustle[20] who reported that rats with fornix transections were unable to remember the appearance of those arms in a 4-armed maze that they had already entered. This task could not be solved by spatial cues as the arms were interchanged after every trial. While there are many differences between this task and that used in the present study, it is hard to identify the critical features responsible for the different outcomes. For example, rats trained in the 4-armed maze received interstimulus intervals of 60 s during acquisition, while those in the present study received delays of almost 0 s. Nevertheless, the normal performance of our HPC animals on the 60 s retention interval suggests that this difference is not crucial. Similarly, the study of Olton and Feustle[20] used a limited set of only 4 stimuli, while the present study used 50 sets of stimuli. This difference was examined in Experiment 5 in which it was found that reducing the number of test stimuli did not differentially affect the HPC animals. Thus there appears to be some other critical factor.

One difference involves the number of items to be retained by the test animals and the number of alternative response choices. In the present experiment the rat only has to remember the last arm and only has two response choices. In the experiment of Olton and Feustle[20] the animal has to remember up to 3 arms and has 4 different response choices. One other possible critical difference is suggested by a recent report on the effects of fornix transection in monkeys, in which Gaffan et al. have argued that the surgery does not interfere with sensory memory, but with the memory of instrumental responses[7]. In the present experiment the rats had merely to decide which arm was unfamiliar and enter it, whereas in Olton and Feustle's experiment[20] all 4 arms were familiar and the rat had to avoid those arms that it had already chosen and entered during the session (i.e. the rats may have to recall their instrumental performance rather than simply identifying as familiar the sensory features of the arm they had visited, and as unfamiliar those of the arms that had not been visited). This argument is contradicted, however, by the finding that hippocampal lesions may impair a recognition task in which the rat may view but not enter the choice boxes, but have no apparent effect when the animal is allowed to enter and explore the boxes[14].

The demonstration that hippocampectomized rats can choose accurately on the present task with retention intervals as long as 60 s conflicts sharply with the recent suggestion that in temporally discontiguous tasks, like the present one, the hippocampus is necessary to maintain a register of recent and relatively recent events[24]. This hypothesis proposes that after hippocampal damage such tasks are soluble only when they can be accomplished by temporary storage of a limited amount of material for a limited time in a residual short-term store. The present design precludes the use of motor strategem for task solution. Hence, given the results of the control experiments, it seems most likely that the hippocampectomized rats were remembering the identity of the sample stimulus during the retention interval. In the present experiment we did not extend the retention interval to an extent that produced a severe drop in response accuracy in normal animals. Nevertheless, the absolute time over which adequate retention was manifest is well outside estimates derived from the performance of rats with almost identical hippocampal lesions in other tasks, such as DRL[4,28] or discrete-trial lever-alternation tasks[27].

At least two other possible explanations of the

impairment remain. The first is that destruction of the hippocampus alone is insufficient to produce a global memory impairment and that for some classes of information a larger system, which includes the hippocampus, needs to be considered. This proposal is supported by studies with monkeys performing an analogous recognition memory task in which combined lesions of the amygdala and hippocampus are necessary to produce a severe memory impairment while hippocampectomy alone is insufficient[3,7,13,15,17]. However, in many other tasks destruction of the hippocampus is sufficient to produce a severe and enduring impairment, as in our Experiment 3. These results indicate that the hippocampus can indeed act as a temporary register for a variety of information but it may only be necessary for some, e.g. spatial or instrumental, information[7,16,18].

A second possibility is that the passage of time per se is not the critical factor determining whether material will be retained in the short-term store, but that a more critical feature is the occurrence of stimuli competing for space in the residual store[10,24]. At present there is little experimental data that bears upon this point, but the results of Experiment 5 demonstrate that there is no general increase in susceptibility to interference in our lesion group. Future experiments may show whether any of the possibilities outlined above merit further consideration.

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