Behavioural Brain Research, 51 (1992) 83–92

BBR 01357

A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory

Abdelkader Ennaceur[a] and Kamel Meliani[b]

[a] *Laboratoire de Psychophysiologie, Université Paris VII, Paris (France) and* [b] *Institut National de Planification et de Statistique, Université Ben Aknoun, Algiers (Algeria)*

(Received 13 February 1991)
(Revised version received 6 February 1992)
(Accepted 14 February 1992)

Key words: Object recognition; Spatial recognition; Working memory; Medial septum; Rat

Rats were submitted to object and spatial recognition tests (both based on the same paradigm) and to the radial-arm maze. The results are as follows: (1) rats could discriminate between a new and a familiar object when the retention delay was 1 min, 15 min or 60 min but not 24 h. The relationship between the level of discrimination and intertrial delays is quadratic with a maximum for 15 min. (2) Exposure to distractive stimuli during the retention delay may impair object recognition. (3) Rats discriminated between a new and a familiar space. (4) There is no correlation between the three tests which argues for a multiple form of working memory, especially a spatial and a non-spatial one. (5) Medial septal lesion did not impair object and spatial recognition memory, but the level of discrimination in the spatial recognition test was significantly reduced compared to that of control.

INTRODUCTION

Analysis of amnesic syndromes put forward two types of memories with different susceptibilities to brain pathology or ageing. The first type is the memory of skills or rules (knowing how) and is spared while the second, the memory of data and events (knowing that), is disturbed. There is no general agreement between authors on the definition of these two kinds of memories. Depending on the authors, the type I (spared) is the semantic, the procedural or the reference memory and the type II (disturbed) is the episodic, the declarative or the working memory[4,7,23,31,45,50]. Only the working memory is satisfactorily reproduced in tests used for animals. Most tests are based on the matching to sample procedure[18], the trial-unique non-matching to sample version is the most popular test presently used in monkeys for the experimental study of amnesic syndromes[27].

However, this test is not purely one-trial since it involves the learning of a rule (matching or non-matching to sample) through the repetition of stimulus or response reward associations. Alterations of performances by drugs or brain lesions may be shown either by effects on the one-trial component or on the learning of a rule, or even both.

A first rat version of the delayed non-matching to sample task[1] suffered from the same drawbacks; in this respect it was not better than the delayed reinforced alternation or the radial maze, the most popular working memory tests in the rat.

In a previous paper[9] we described another rat version of the Mishkin and Delacour test which is really a 'pure' working memory task: for it does not involve at all the learning of a rule, since it is based entirely on the spontaneous exploratory behavior of rats toward objects. This factor is of great importance if we consider that the critical factor in the pathology of memory is repetition. Memory of a unique event is known to be more vulnerable than that based on the repetition of some conditions, such as the associations of stimulus or responses to a reinforcer. Moreover, the fact of not being based on primary positive or negative reinforcers such as electric shocks or food has another advantage. Compared to most other models, in our test the arousal

Correspondence: A. Ennaceur, University of Durham, Department of Psychology, Science Laboratories, South Road, Durham, DH1 3LE, UK.

and motivational states of animals are much more similar to those under which the human memory is usually measured.

This test has proved to be sensitive to various pharmacological and neurological treatments[8,10–12]. The purpose of the experiments reported here was to further its behavioral analysis on four points: (1) the relationship between object recognition and the duration of the retention delay; (2) the effect of distractive stimuli during the retention delay; (3) the relationships between object recognition, spatial recognition (both based on the same procedure) and the radial maze learning. The distinction between spatial vs. non-spatial components seems to be crucial for the description of normal as well as disturbed memory[2,5,13,16,21,35–37,39] and (4) the sensitivity of spatial vs. non-spatial recognition memory to the effect of medial septal lesion in rats. This latter point is raised by some reports on the effect of the lesion of the septo-hippocampal system or ageing on both memory forms[5,12,13,16,38,40,47].

EXPERIMENT 1

Methods

Subjects

Forty-one male Wistar rats weighing 200–250 g each were used. They were housed in individual cages and maintained on a 12–12 h light/dark cycle (07.00–19.00 h). Ambient temperature was 23 °C ± 1. During the entire experiment they had free access to food and water.

Apparatus

The apparatus was an open box made of wood 65 × 45 × 45 cm (height). The objects to be discriminated were made of glass, plastic or metal and existed in duplicate. Their weight was such that they could not be displaced by rats. The apparatus was placed in a sound-isolated room where the sound-insulation was aided by a masking-white noise of 70 dB above the human threshold. A light bulb fastened in the upper part of the room provided a constant illumination of about 40 lux at the level of the test apparatus.

Behavioural testing

The methods are fully explained in a previous paper[9]. They may be summarized as follows: all rats were submitted to two habituation sessions where they were allowed 3 min to explore the apparatus. Twenty-four h later, testing began. This experiment comprised ten sessions of 5 different intertrial conditions separated by 48 h each: 2 sessions with 1-min, 15-min, 60-min and 24-h intertrial delays and 2 sessions where distractive stimuli were introduced during a 15-min intertrial delay. Each session was made of two trials. In the first trial (T1), rats were exposed to two identical objects which constituted samples A1 and A2. In the second trial (T2), the rats were exposed again to two objects, one of the samples which was then the familiar object A and a new object B. In each trial, each object was placed in one of the back corners of the box. The object A presented during T2 was a duplicate of the sample presented in T1 in order to avoid olfactory trails. From rat to rat, the role (sample or new object) as well as the position of the two objects were counterbalanced and randomly permuted during T2. A different pair of objects was used for each session. Let's call *O1* and *O2*, the two objects of each pair. For half of the rats, *O1* was the sample A and *O2* the new object B. For the other half, the role of *O1* and *O2* was inversed. These precautions were taken in order to reduce object and place preference effects. It should be stressed that the objects apparently had no natural significance for rats; they had never been associated with a reinforcement. The duration of T1 and T2 was of 3 min.

The rats were submitted in a counterbalanced sequence to each delay condition. In each condition, rats remained in their cages in the testing room until they completed the second trial except for the 24-h delay: in this case they were returned to the animal room. In the distractive stimuli condition, rats were placed in a new environment for a 15-min intertrial delay; in this new environment, they were allowed to explore a new object which differed from A and B by the fact that it could be moved by rats.

Measurements and statistical treatments

The basic measure was the time spent by rats in exploring objects during T1 and during T2. Exploration of an object was defined as follows: directing the nose to the object at a distance ≤2 cm and/or touching it with the nose; turning around or sitting on the object was not considered as exploratory behavior.

Let's call *a1* and *a2* the time spent in exploring the two identical samples in T1; and *a* and *b* the time spent in T2 in exploring respectively the sample and the new object. From these basic measures, the following variables were considered: *e1*, *e2*, *h*, *d* (see Table 1).

e1 and *e2* measure the total exploration of both objects during T1 and T2 respectively, *h* may be considered as a measure of habituation of the exploratory behavior; *d* as a mnesic measure reflecting the discrimination between the new and the familiar object.

TABLE I

Index of the different measures involved in the object recognition test

e1 is the measure of the time spent by rats in exploring both samples (*a1* and *a2*) in the acquisition trial and *e2* is the measure of the time spent by rats in exploring both objects (*a*: familiar object, and *b*: new object) in the retention trial. *h* is the measure of global habituation from trial 1 (acquisition trial) to trial 2 (retention trial) and *d* is the measure of discrimination between new and familiar objects.

Exploration	*Habituation*	*Discrimination*
$e1 = a1 + a2$ $e2 = a + b$	$h = e1 - e2$	$d = b - a$

Wilcoxon test was used on variables *h* and *d*. For instance, significance of *h* for a given intertrial delay condition was tested by comparing the mean value of *e1* and *e2* for the group. Comparisons between different experimental conditions were based on Friedman test and post-hoc comparisons according to Siegel and Castellan[43]. Linear, quadratic and cubic components of the object recognition–retention delay relationship were evaluated by trend analysis according to Winer[49]. Correlations between different variables within the same condition were also computed.

Results

Overall exploratory activity

As shown in Fig. 1, *e1* remained stable through sessions. On the contrary, *e2* significantly varied according to the intertrial delay ($F_{5,41} = 66.83$, $P \leq 0.01$). The level of exploration *e2* was higher after 15 min and 60 min delay than after 1 min or after exposure to distractive stimuli ($P \leq 0.01$).

Overall habituation (h)

Overall habituation (h) was significant in the 1-min, 24-h and distractive stimuli condition ($P \leq 0.01$) but not in the 15-min and 60-min delay conditions. Comparisons between intertrial conditions showed (Fig. 2) that the level of overall habituation was significantly higher in the 1-min, 24-h and distractive conditions than in the 15-min and 60-min conditions. Tests for trend showed that the relationship between *h* and the intertrial delays had significant linear ($F_{1,120} = 14.69$, $P \leq 0.01$) and quadratic ($F_{1,120} = 26.14$, $P \leq 0.01$) components.

Object recognition (d) in the different conditions (Fig. 3)

Rats discriminated between the new and the familiar object after 1-min, 15-min and 60-min delay conditions ($P \leq 0.01$). On the contrary, discrimination scores were not significant in the 24-h delay and after exposure to distractive stimuli conditions ($P \geq 0.10$). Between condition test revealed that *d* was significantly inferior in 24-h delay and distractive stimuli conditions when compared to the other conditions. Tests for trend showed that the relationship between *d* and the intertrial delays had not only a significant linear ($F_{1,120} = 13.75$, $P \leq 0.01$) but also significant quadratic ($F_{1,120} = 33.37$, $P \leq 0.01$) components.

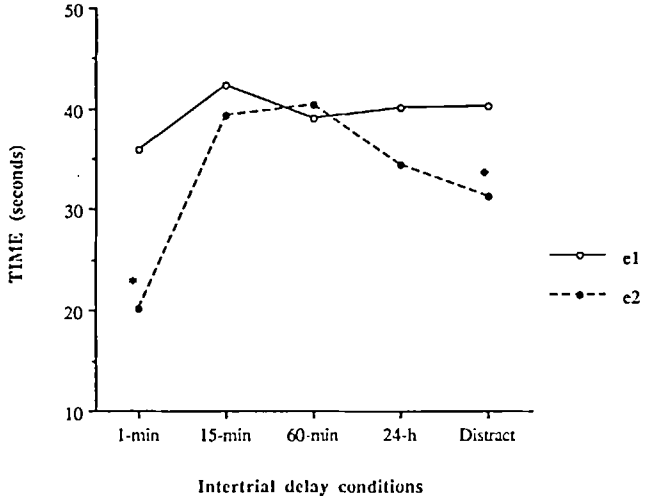

Fig. 1. Mean value of the total exploration time spent by rats on both sample objects in trial 1 and on both new and familiar objects in trial 2 of each delay condition. Friedman test on the differences between the different intertrial delays: * compared to each delay condition, $P \leq 0.01$; and ◆ compared to 15- and 60-min delay conditions, $P \leq 0.01$.

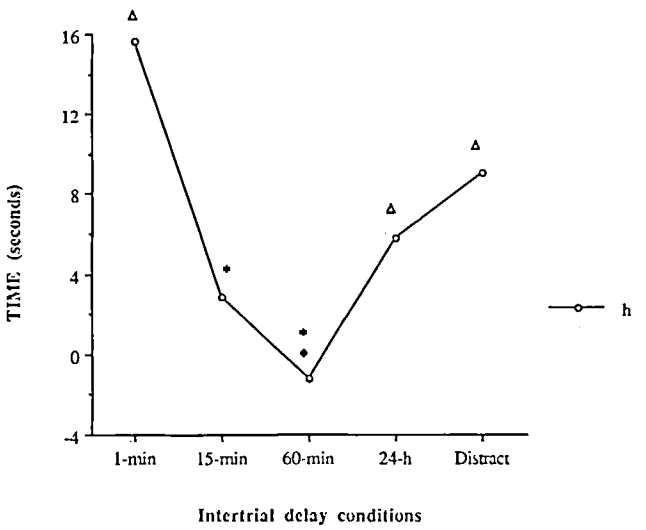

Fig. 2. Mean value of the index of habituation *h* of each delay condition. Friedman test on the differences between the different intertrial delays: * compared to 1-min delay condition, $P \leq 0.01$; ◆ compared to distractive delay condition, $P \leq 0.01$; △ Wilcoxon test on within delay condition, $P \leq 0.01$.

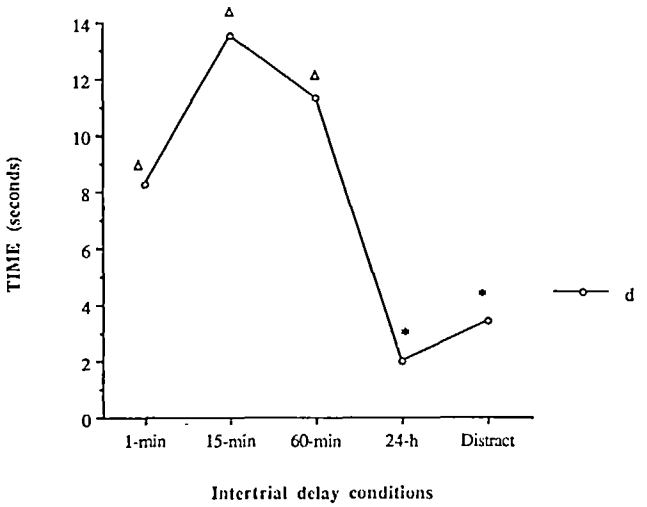

Fig. 3. Mean value of the index of discrimination *d* of each delay condition. Friedman test on the differences between the different intertrial delays: * compared to 1-min, 15-min and 60-min delay conditions, $P \leq 0.01$; $^{\Delta}$ Wilcoxon test on within delay condition, $P \leq 0.01$.

Correlation between variables in each delay condition

Measures of exploration *e1* and *e2* were positively and significantly correlated in 1-min ($r = 0.48$, $P \leq 0.01$), 60-min ($r = 0.36$, $P \leq 0.02$), 24-h ($r = 0.60$, $P \leq 0.01$) and distractive stimuli conditions ($r = 0.39$, $P \leq 0.01$) but not in 15-min delay condition. Habituation measure *h* was correlated with *e1* ($r \geq 0.40$, $P \leq 0.01$) and negatively with *e2* ($r \geq 0.30$, $P \leq 0.05$) in all conditions. The level of object recognition *d* correlated significantly and positively to the measure of exploration *e1* in 1-min delay condition ($r = 0.47$, $P \leq 0.01$) and to *e2* in 1-min ($r = 0.56$, $P \leq 0.01$), 15-min ($r = 0.63$, $P \leq 0.01$) and 60-min ($r = 0.50$, $P \leq 0.01$) delay conditions. It correlated negatively and significantly to the measure of habituation *h* in 15-min ($r = 0.53$, $P \leq 0.01$), 60-min ($r = 0.32$, $P \leq 0.01$) and positively to the distractive stimuli condition ($r = 0.42$, $P \leq 0.01$).

EXPERIMENT 2

Methods

Subjects

The same rats as in Expt. 1.

Apparatus

The previously described open box in Expt. 1 was used, but it was divided in 3 compartments of equal size. The central part constituted the start-box from which the rat could enter either side compartments. The experiment was conducted in the same experimental room as Expt. 1.

Behavioural testing

Forty-eight h after completion of Expt. 1, rats were submitted to place recognition testing. In T1, only one compartment was open, on the contrary in T2 rats were allowed to explore both compartments, that open in T1 (familiar compartment, the equivalent of the sample object in the object recognition test) and that previously closed (the new compartment). There were no differences between the two compartments and no cues. Intertrial delay was 15-min long.

Measurements and statistical treatments

The basic measure was the time spent by rats in exploring either compartments during T1 and during T2. An exploration episode started each time the head of the rat crossed the opening between the starting box and a compartment. From exploration measures, variables *e1*, *e2* and *d* defined as in Expt. 1, were computed. *d* was submitted to Wilcoxon t-test. The value scores are presented by means (m) and S.E.M.'s.

Results

Rats spent significantly more time in exploring the new compartment ($m = 84.02 \pm 2.06$) than the familiar one (54.12 ± 1.85). There was no significant correlation between *d* and *e1* or *e2* measures.

EXPERIMENT 3

Methods

Subjects

The same rats as in Expt. 1.

Apparatus

It consisted in an elevated eight-arm radial-maze made of 0.5 cm black plexiglass. Each arm (68 × 10 cm) extended from an octogonnally shaped central hub (30 cm in diameter). Sidewalls (1.5 cm high) extended the length of each arm. A small cup (4 cm) was placed at the end of each arm. The maze was elevated 80 cm above the floor and placed in a sound attenuated room with a masking noise of 70 dB above the human threshold. Several large and distinctive objects having a constant location in the room were placed as extramaze cues.

Habituation

Forty-eight h after completion of Expt. 2, rats were submitted to three habituation sessions (one per day) in the radial-maze. Rats were put on the central plateform

and were allowed to explore the entire maze for 10 min. During this period, rats had free access to food and water.

Training

Rats were given twelve sessions (one per day). A session lasted until the rat made eight choices or 10 min elapsed. The cups at the end of each arm contained 0.3 ml of water. The experimenter recorded the order of the eight choices. One hour after the completion of the session rats were given water for 10–15 min.

Measurements and statistical testing

Five measures are considered: (1) Mean number of errors per session (ER): an error was an entrance in a previously visited arm. (2) Mean number of correct choices before the first error in each session (ERS). (3) Mean number of errors and sessions to criterion (EC, SC): the acquisition criterion was one error or less per session during five consecutive sessions. (4) Measure of sequential response strategy (SRC): SRC consists in choosing successively the immediate neighbour of the last visited arm in a constant direction: clockwise or the reverse. SRC may facilitate performances: moreover, it involves a reference memory (that of the rule described above) more than a working memory which makes difficult the interpretation of results. It was measured as follows: for each choice, from the second to the 8th, the score was 1 (respectively −1) if the arm chosen at choice n was the immediate clockwise (respectively −1 counterclockwise) neighbour of that chosen at choice $n-1$, otherwise the score was zero. In spite of some biases, the total score for a session reflects the importance of the SRC: absolute scores of 7 reflect a perfect sequential strategy whereas those near zero a non-sequential order of choices. (5) Mean time spent by rats to perform eight choices within each session (TIM).

Correlations between these different measures were computed. The value scores are presented by means (m) and S.E.M.'s.

Results

The mean values ($\pm$ S.E.M.) of the different measures involved in the radial-maze are as follows: the mean total number of errors (ER, $m = 1.32 \pm 0.06$), the mean total number of correct choices before first errors (ERS, $m = 5.33 \pm 0.13$), the mean total number of errors (EC, $m = 9.71 \pm 1.05$) and sessions (SC, $m = 5.63 \pm 0.83$) to reach criterion; the mean total number of sequential response choices (SRC, $m = 1.44 \pm 0.15$) and the mean total time spent by rats in performing 8 choices (TIM, $m = 192.53 \pm 9.69$).

Correlation between the different measures involved in radial-maze

ER, EC, SC are significantly and positively intercorrelated to each other ($P \leq 0.01$) and negatively correlated to ERS ($P \leq 0.01$) (see Table II) but the mean time spent by rats to make 8 choices as well as measures of SRC do not correlate to any other measures.

Correlations between memory measures of the three tests

As shown in Table II, significant correlations are mostly between measures from the same tests. The only three significant intertest correlations (d in 24-h delay condition with d in place recognition condition, d in distractive condition with total number of correct responses before first error and with the number of session to reach criterion in radial-maze) are difficult to

TABLE II

Correlations between memory measures involved in object or spatial recognition and radial maze tests

Conditions	*1 min*	*15 min*	*60 min*	*24 h*	*Distract*	*Place*	*ER*	*ERS*	*EC*
15 min	0.04								
60 min	0.37*	0.20							
24 h	0.14	−0.05	−0.28						
Distract	−0.12	−0.15	0.19	0.01					
Place	−0.01	−0.18	−0.22	0.43*	0.03				
ER	0.06	−0.03	−0.04	0.05	0.20	−0.05			
ERS	0.12	−0.02	0.13	−0.10	−0.35*	0.06	−0.75*		
EC	0.01	−0.06	−0.04	0.11	0.30*	−0.07	0.94*	−0.75*	
SC	−0.02	−0.02	−0.03	0.11	0.35*	−0.01	0.85*	−0.73*	0.95*

* $P \leq 0.05$.

interpret since from within condition tests object recognition was not significant after 24-h delay or distractive stimuli.

EXPERIMENT 4

Methods

Subjects

The same rats as in Expt. 1.

Surgeries

All surgery was performed under pentobarbital sodium anesthesia. The rats were placed in a stereotaxic instrument (Horsey-Clark). Lesions were produced electrolytically by passing a DC cathodic current (1.0 mA for 20 s) through a 0.14-mm-diameter stainless steel electrode that was inserted stereotaxically into the brain. Coordinates for the unilateral lesion were taken from the atlas of Albe Fessard et al.[3] and applied on medial septal nuclei (MS) for 23 rats (9.0 mm anterior, 0.0 mm lateral to lambda and 6.5 ventral to the skull surface), the electrode on sham-control rats ($n = 18$) was stoped at the corpus callosum level.

Histology

At the completion of the experiments, the lesioned animals as well as operated control were deeply anaesthetized and transcardially perfused with 10% formalin. The brains were removed and stored in formalin for about 8 days, then frozen and sectioned at 100 microns. The level considered was at MS structures for visualising the site of the lesion. After this histological check, some experimental animals were discarded.

EXPERIMENT 4A

Methods

Apparatus

See Expt. 1.

Behavioural testing

See Expt. 1. Thirty days after surgery, rats were submitted to one habituation session where they were allowed 3 min to explore the apparatus. Twenty-four h later, testing began. The rats were submitted in a counterbalanced sequence to each delay condition. In each test condition, rats were submitted to 2 sessions separated by 48 h each. The duration of T1 and T2 was 3 min each and the delay between them was 15 min and 60 min. In the distractive stimuli condition, rats were placed in new environment for a 15-min intertrial delay; in this new environment, they were allowed to explore a new object which differed from A and B by the fact that it could be displaced by rats.

Measurements and statistical treatments

(a) Wilcoxon test was performed within each group on each session for *h* and *d* measures and between sessions for all measures of the same intertrial conditions. When there were no significant differences between the two sessions of the same intertrial conditions the data were pooled within each group.

(b) Friedman test was performed within group between the different intertrial conditions for all measures and post-hoc comparisons according to Siegel and Castellan[43].

(c) For comparisons between control and medial septal lesioned rats we used the Mann–Whitney *U*-test on measures of the same intertrial conditions.

Results

Histology

The lesions extended ventral to the level of the anterior commissure and rostrocaudally to a level anterior to the postcommissural fornix. All selected rats had lesions of the medial septal nuclei and the dorsal part of the vertical arms of the diagonal band. Most rats evidenced damage to the intermediate portion of the lateral septal nucleus, the septo-hippocampal nucleus and the fornix. Three rats were excluded after a histological examination (see Fig. 4).

Overall exploration

Since performance of rats on *e1* remained stable throughout sessions their results were pooled. On the contrary *e2* varied significantly according to the intertrial delay conditions in control ($F_{3,18} = 6.12$, $P \leq 0.05$) and in septal ($F_{3,20} = 12.18$, $P \leq 0.01$) groups: the level of exploration *e2* was higher after 15 min and 60 min than after distractive stimuli condition in both groups ($P \leq 0.05$). Comparisons between groups on *e1* and *e2* measures showed that septal rats explored more than control in retention trial *e2* in each intertrial condition ($P \leq 0.05$), see Fig. 5.

Global habituation (h)

Within group tests for each delay. When the delay was 15 min, MS group showed a significant increase of exploration activity in T2 compared to T1 ($P \leq 0.01$). Under intertrial distractive conditions C and MS

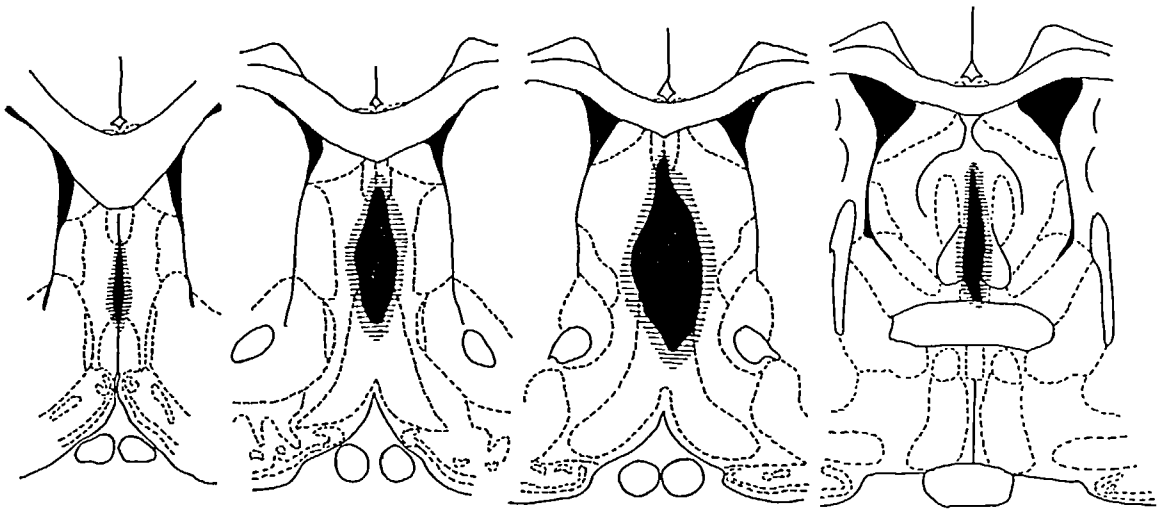

Fig. 4. Representative examples of medial septal lesions according to the atlas of Paxinos and Watson[34]. The cross-hatched areas shows the maximum extent of the lesions and the filled area shows the common extent.

groups showed a significant decrease of their exploratory activity in T2 compared to T1 ($P \leq 0.01$).

Within group tests of the influence of delays. The level of habituation was significantly different between intertrial conditions in C ($F_{3,18} = 8.67$, $P \leq 0.01$) and in MS ($F_{3,18} = 19.17$, $P \leq 0.01$) groups: the level of habituation was significantly higher after the distractive stimuli condition than after 15 min and 60 min ($P \leq 0.01$).

Between group tests. There were significant differences between groups when the intertrial delay was 15 min ($P \leq 0.01$). (Fig. 6.)

Discrimination between the familiar and the new object (d)

Within group tests for each delay. Rats in each group spent significantly more time in exploring a new object than a familiar one when the intertrial conditions was 15 min and 60 min ($P \leq 0.01$), but not under distractive conditions.

Within group tests of the influence of delays. The level of discrimination was significantly different between intertrial conditions in MS group ($F_{3,18} = 14.40$, $P \leq 0.01$): the level of discrimination was significantly lower after distractive stimuli condition than after 15 min delay ($P \leq 0.01$).

Between group tests. The level of discrimination *d* was higher in MS than in C group when intertrial delay was 15 min ($P \leq 0.01$) and 60 min ($P < 0.05$) but not after distractive conditions ($P > 0.10$).

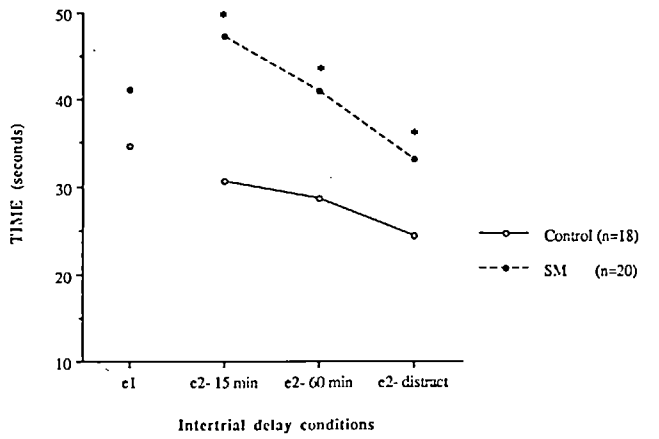

Fig. 5. Mean value of the total exploration time spent on sample objects in trial 1 (*e1*) and on both new and familiar objects in trial 2 (*e2*) of each delay condition in control and SM groups. * Compared to control, $P \leq 0.01$.

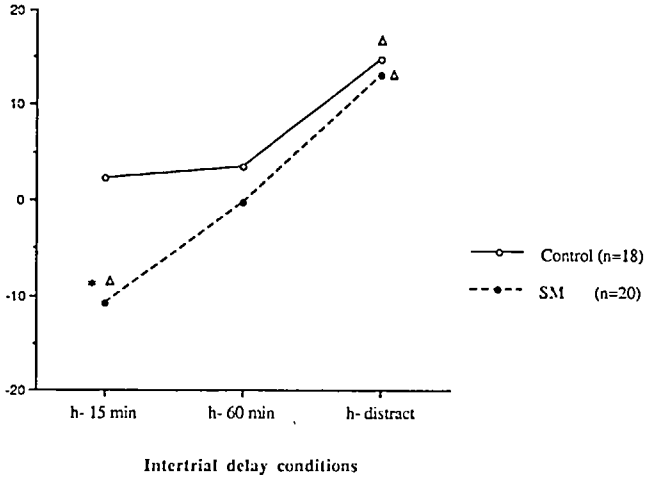

Fig. 6. Mean value of the index of habituation *h* of each delay condition in control and SM groups. * Compared to control, $P \leq 0.01$. $^{\Delta}$ Wilcoxon test on within delay condition, $P \leq 0.01$.

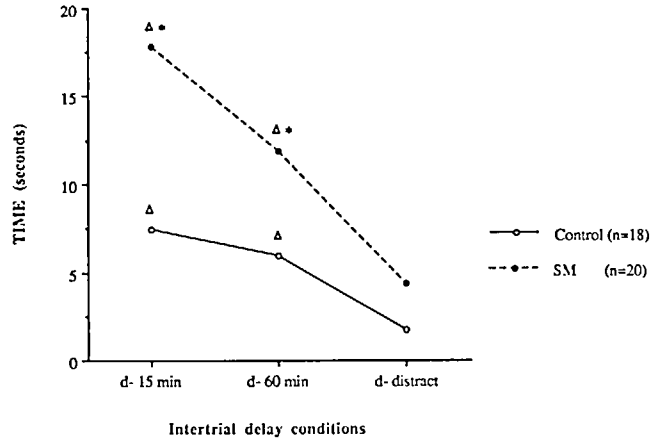

Fig. 7. Mean value of the index of discrimination *d* of each delay condition in control and SM groups. * Compared to control, $P \leq 0.01$. $^{\Delta}$ Wilcoxon test on within group and delay condition, $P \leq 0.01$.

EXPERIMENT 4B

Methods

Subjects

The same rats as in Expt. 1.

Apparatus and behavioural testing

See Expt. 2.

Measurements and statistical treatments

Variables *e1*, *e2* and *d* defined as in Expt. 1 were computed and submitted to Mann–Whitney *U*-test for between group comparisons, and *d* was submitted to Wilcoxon test for within group comparison of the time spent by rats in exploring the familiar and the new compartments. The value scores are represented by means (m) and S.E.M.'s.

Results

Overall exploratory activity

The level of exploration *e1* (C, m = 74.39 ± 3.37 and SM, m = 79.15 ± 3.07) and *e2* (C, m = 118.06 ± 4.03 and SM, m = 129.85 ± 4.08) were comparable for the two groups ($P \geq 0.10$).

Discrimination between a new and a familiar compartment

Both groups spent more time in exploring the new compartment than the familiar one (C, T = 3, $P \leq 0.01$; MS, T = 20.5, $P \leq 0.01$), however the level of discrimination was significantly higher ($P \leq 0.05$) in C (m = 35.5 ± 5.34) compared to that of MS group (21.25 ± 5.38).

DISCUSSION

Our results give indications on the following points.

The effect of the length of the retention delay and interpolated distractive stimuli on object recognition

Rats discriminate between a familiar and a new object after a delay of 1 min, 15 min and 60 min but not after a 24-h delay. Exposure to a distractive stimuli during the retention delay impaired object recognition. The relationship between object recognition (as measured by *d*) and the retention delay has a significant quadratic component, with the greatest point for the 15-min delay. Several explanations are possible: the strength of the 'trace' laid by the sample in trial 1 may evolve according to a non-monotonous function[44], but *d* more likely reflects the interaction of several factors: for instance, the strength of the 'trace' of the sample in trial 2, and a retrieval factor such as the level of arousal in the trial 2. This level, insofar it is measured by *e2* — the total exploration of both objects in trial 2 — was significantly lower after a 1-min delay than after a 15-min or 60-min delay (see Results of Expt. 1). A difference in the level of arousal in trial 2, in the 1-min condition on one side and the 15-min and 60-min on the other, is also suggested by the fact that there is a significant global habituation of the exploration of objects (*h*) from T1 and T2 in the 1-min delay but not in the 15-min and 60-min delay conditions. Moreover intercondition tests showed that *h* was significantly higher in 1-min delay than in 15-min and 60-min delays. In this respect it should be noted that *h* was also significant in two conditions where there was no object recognition: after 24-h delay or after a distractive stimuli. However in these cases, the poor memory performance was probably due either to the 'trace' factor (insufficient strength or alteration by retroactive interference) or to a retrieval factor such as proactive interference rather than to a low arousal level.

d does not correlate to *e1*, total exploration during the acquisition trial, but to *e2*, total exploration during the retention trial. The positive and significant correlation of *d* to *e2*, is not trivial: it is conceivable that high (respectively low) scores of total exploration of objects could be associated to low (respectively high) scores of discrimination between new and familiar objects.

The possibility of a 'pure' spatial working memory test

Rats made a good performance in the place recognition test based on the same procedure as in object recognition. Further research will compare place and object recognition after different intertrial delays and after distractive stimuli. The apparatus used here does

not permit parametric studies on the spatial discrimination performance of rats but we are in the way to present a test which should allow testing rats for more than one session in the same condition.

The relationships between measures of spatial vs. non-spatial working memory tests in rats

There were no significant correlations between mnesic measures from the three tests. The lack of correlations between the object and place recognition, in spite of the fact that the procedure and the experimental situation were identical, is a strong argument in favor of the existence of two forms of working memory in rats: spatial and non-spatial. Lack of correlations between either object or place recognition and the radial maze is more difficult to interpret — place recognition and radial maze tests set into play spatial information — however they differ by several aspects: the amount of information, probably larger in the radial-maze (8 places or cues) than in the recognition tests (2 places or 2 objects); the relative weight of distant vs. local cues, the former being probably more important in the radial maze; a use of reinforcer and significant reference memory component in the radial maze, completely absent in the object and place recognition tests.

The differential effect of septal lesion on measures of spatial vs. non-spatial working memory tests in rats

Rats with lesion of the medial septum discriminated between new and familiar objects as well as between new and familiar compartments, but the level of discrimination was significantly lower in septal lesioned rats compared to control in the spatial recognition test. In object recognition test septal lesion did not disturb discrimination, it even enhanced it, but in the place recognition test the level of discrimination was reduced.

The results of our experiments suggest that there are at least two forms of working memory (the spatial and non-spatial one) but the effect of medial septal lesion on place recognition is not clear-cut. The hypothesis that there are two forms of working memory is supported by a lack of correlation between mnesic measures involved in object and spatial recognition tests. Many reports investigating the role of the septo-hippocampal formation have demonstrated such dissociation, but there is no general agreements between authors according to the behavioral tasks and procedure involved. Olton and Freustle[32] and Raffaele and Olton[37] suggest that damage of the septo-hippocampal system disrupts the working memory in radial maze irrespective of the spatial requirements of the test employed, whereas Aggleton and colleagues[2] emphasize the spatial nature of the deficit produced by hippocampal damage which did not disrupt a non-spatial working memory in a Y-maze. In agreement with the latter hypothesis it has been reported that lesion of the hippocampus and its connections impair spatial memory in various tests in rats and monkeys[2,14,25,26,28,30,33,35,46,48] but not object, visual, tactile or olfactory recognition or discrimination[6,17,19,24,25,29,35,41,42] and this impairment is reversed or attenuated by the introduction of cues[20,22,25,29,30,40]. In experiments conducted in our laboratory[11], we observed that the low doses of scopolamine (0.25 and 0.50 mg/kg i.p.) which disturbed radial maze learning were without effect on object-recognition memory of rats. Accordingly, we suggest that the spatial recognition is more vulnerable than the object recognition because it is based on a less redundant information: the various features of the object are probably coded on more dimensions than a place.

In conclusion, our recognition procedures offer interesting characteristics for devising experimental models of amnesia in rats: (1) 'pure' measures of working memory. (2) Sensitivity to retention delay and distraction which confirm and extend the results previously reported[9]. (3) Rather selective and comparable measures of spatial or non-spatial forms of memory. (4) Sensitivity to amnestic or promnesic treatments[8,10–12].

On all these points our tests seem to compare favourably with those currently used in the field of the neurobiology of learning and memory.

ACKNOWLEDGEMENTS

We thank Susan Mary Walker for the editing of this paper.

REFERENCES

1 Aggleton, J.P., One-trial object recognition by rat, *Quart. J. Exp. Psychol.* 37B (1985) 279–294.
2 Aggleton, J.P., Hunt, P.R. and Rawlins, J.N.P., The effects of hippocampal lesions upon spatial and non spatial tests of working memory, *Behav. Brain Res.*, 19 (1986) 133–146.
3 Albe-Fessard, D., Stutinsky, F. and Libouban, S., *Atlas stereotaxique du Diencéphale du Rat blanc*, Editions du CNRS, Paris, 1971.
4 Baddeley, A., Amnesia: a minimal model and an interpretation. In L.S. Cermak, (Ed.), *Human Memory and Amnesia*, Erlbaum, Hillsdale, 1982, pp. 305–336.
5 Beatty, W.W., Preservation and loss of spatial memory in aged rats and humans: implications for the analysis of memory dysfunction in dementia, *Neurobiol. Aging*, 9 (1988) 557–561.
6 Brito, G.N.O. and Brito, L.S.O., Septohippocampal system and the prelimbic section of frontal cortex: a neuropsychological battery analysis in the rat, *Behav. Brain Res.*, 10 (1990) 127–146.
7 Cermak, L.S., The episodic-semantic distinction in amnesia. In L.S. Cermak, (Ed), *Human Memory and Amnesia*, Erlbaum, Hillsdale, 1984, pp. 55–62.

8 Ennaceur, A. and Delacour, J., Effect of combined or separate administration of piracetam and choline on learning and memory, *Psychopharmacology*, 92 (1987) 58–67.

9 Ennaceur, A. and Delacour, J., A new one-trial test for neurobiological studies of memory in rats. I. Behavioral data, *Behav. Brain Res.*, 31 (1988) 47–59.

10 Ennaceur, A. and Delacour, J., A new one-trial test for neurobiological studies of memory in rats. II. Effects of piracetam and pramiracetam, *Behav. Brain Res.*, 33 (1989) 197–207.

11 Ennaceur, A. and Meliani, K., Effects of physostigmine and scopolamine on rats' performances in object recognition and radial-maze tests, *Psychopharmacology*, in press.

12 Ennaceur, A., Lesions of cholinergic structures on working memory in rats and the effects of physostigmine and amphetamine, *14th Annual Meeting of the ENA, Cambridge, 8–12th September 1991*, Oxford Univ. Press, 1991.

13 Flicker, C., Bartus R.T., Crook, T.H. and Ferris, S.H., Effects of aging and dementia upon recent visuospatial memory, *Neurobiol. Aging*, 5 (1984) 275–283.

14 Gaffan, D. and Harrison, S., A comparison of the effects of fornix transection and sulcus principalis ablation upon spatial learning by monkeys, *Behav. Brain Res.*, 31 (1989) 207–220.

15 Gaffan, D. and Harrison, S., Reversal learning by fornix-transected monkeys, *Quat. J. Exp. Psychol.*, 36B (1984) 223–234.

16 Gallagher, M. and Pelleymounter, M.A., Spatial learning deficits in old rats: a model for memory decline in the aged, *Neurobiol. Aging*, 9 (1988) 549–556.

17 Gray, D.S. and Sainsbury, R.S., The effects of fimbria lesions on cue utilization in the rat, *Physiol. Behav.*, 22 (1979) 325–329.

18 Honig, W.A., Studies of working memory in the pigeons. In S.H. Hulse, H. Fowler and W.K. Honig (Eds.), *Cognitive Processes in Animal Behavior*, Hillsdale, Erlbaum, 1978, pp. 211–248.

19 Hunter, N. and Murray, C.L., Cholinergic mechanisms in a simple test of olfactory learning in the rat, *Psychopharmacology*, 99 (1989) 270–275.

20 Jarrard, L.E., Selective hippocampal lesions and behavior: effects of kainic acid lesions on performance of place and cue tasks, *Behav. Neurosci.*, 97 (1983) 873–889.

21 Johnson, M.K. and Hasher, L., Human learning and memory, *Annu. Rev. Psychol.*, 38 (1987) 631–668.

22 Kelsey, J.E. and Landry, B.A., Medial septal lesions disrupt spatial mapping ability in rats, *Behav. Neurosci.*, 102 (1988) 289–293.

23 Kinsbourne, M. and Wood, F., Theoretical considerations regarding the episodic-semantic memory distinction. In L.S. Cermak, (Ed.), *Human Memory and Amnesia*, Erlbaum, Hillsdale, 1982, pp. 195–218.

24 Kohler, C and Srebo, B., Effects of lateral and medial septal lesions on exploratory behavior in the albino rat. *Brain Res.*, 182 (1980) 423–440.

25 Mahut, H., A selective spatial deficit in monkeys after transection of the fornix, *Neuropsychologia*, 10 (1972) 65–74.

26 Markowska, A.L., Olton, D.S., Murray, E.A. and Gaffan D., A comparative analysis of the role of fornix and cingulate cortex in memory: rats, *Exp. Brain Res.*, 74 (1989) 187–201.

27 Mishkin, M. and Delacour, J., An analysis of short term visual memory in the monkey, *J. Exp. Psychol., Anim. Behav. Processes*, 1 (1975) 326–334.

28 Morris, R.G.M., Garrud, P., Rawlins, J.N.P. and O'Keefe, J., Place navigation impaired in rats with hippocampal lesions, *Nature*, 297 (1982) 681–683.

29 Murray, E.A., Davidson, M., Gaffan, D., Olton, D.S. and Suomi, S., Effects of fornix transection and cingulate cortical ablation on spatial memory in rhesus monkeys, *Exp. Brain Res.*, 74 (1989) 173–186.

30 O'Keefe, J. and Nadel, L., *The Hippocampus as a Cognitive Map*, Clarendon Press, Oxford, 1978.

31 Olton, D.S., Memory functions and the hippocampus. In W. Seifert (Ed.), *Neurobiology of the Hippocampus*, Academic Press, New York, 1983, pp. 335–373.

32 Olton, D.S. and Freustle, W.A., Hippocampal function required for non-spatial working memory, *Exp. Brain Res.*, 41 (1981) 380–391.

33 Olton, D.S. and Werz M.A., Hippocampal function and behavior: spatial discrimination and response inhibition, *Physiol. Behav.*, 20 (1978) 597–607.

34 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, New York, 1982.

35 Peinado-Manzano, M.A., The role of the amygdala and the hippocampus in working memory for spatial and non-spatial information, *Behav. Brain Res.*, 38 (1990) 117–134.

36 Pelleymounter, M.A., Smith, M.Y. and Gallagher, M., Spatial learning impairments in aged rats trained with salient configuration of stimuli, *Psychobiology*, 15 (1987) 248–254.

37 Raffaele, K.C. and Olton, D.S., Hippocampal and amygdaloid involvement in working memory for non spatial stimuli, *Behav. Neurosci.*, 102 (1988) 349–355.

38 Rapp, P.R. and Amaral D.G., Evidence for task dependent memory dysfunction in the aged monkey, *J. Neurosci.*, 9 (1989) 3568–3576.

39 Rapp, P.R., Rosenberg, R. and Gallagher, M., An evaluation of spatial information processing in the aged rat, *Behav. Neurosci.*, 101 (1987) 3–12.

40 Ridley, R.M., Samson N.A., Baker, H.F. and Johnson, J.A., Visuospatial learning impairments following lesion of the cholinergic projection to the hippocampus, *Brain Res.*, 456 (1988) 71–87.

41 Ridley, R.M., Aitken, D.M. and Baker, H.F., Learning about rules but not about reward is impaired following lesions of the cholinergic projection to the hippocampus, *Brain Res.*, 502 (1989) 306–318.

42 Rothblat, L.A. and Kromer, L.F., Object recognition memory in the rat: the role of the hippocampus, *Behav. Brain Res.*, 42 (1991) 25–32.

43 Siegel, S. and Castellan, N.J., *Nonparametric Statistics for the Behavioral Sciences*, McGraw Hill, New York, 1988.

44 Spear, N.E., *The Processing of Memories: Forgetting and Retention*, Erlbaum, Hillsdale, NJ, USA, 1978.

45 Squire, L.R. and Cohen, N.J., Human memory and amnesia. In G. Lynch, J.L. McGaugh and N.M. Weinberger, (Eds.), *Neurobiology of Learning and Memory*, The Guilford Press, New York, 1984, pp. 3–64.

46 Sutherland, R.J., Whishaw, I.Q. and Kolb, B., A behavioural analysis of spatial localization following electrolytic, kainate or colchicine induced damage to the hippocampal formation in the rat, *Behav. Brain Res.*, 7 (1983) 133–153.

47 Walker, L.C., Kitt, C.A., Struble, R.G., Wagster, M.V., Price, D.L. and Cork, L.C., The neural basis of memory decline in aged monkeys, *Neurobiol. Aging*, 9 (1988) 657–666.

48 Whishaw, I.Q., Cholinergic receptor blockade in the rat impairs locale but not taxon strategies for place navigation in a swimming pool, *Behav. Neurosci.*, 99 (1985) 979–1005.

49 Winer, B.J., *Statistical Principles in Experimental Design*, McGraw Hill, New York, 1971.

50 Wood, F., Ebert, V. and Kinsbourne, M., The episodic-semantic memory distinction in memory and amnesia: clinical and experimental observations. In L.S. Cermak (Ed.), *Human Memory and Amnesia*, Erlbaum, Hillsdale, 1982, pp. 167–194.