Behavioural Brain Research, 31 (1988) 47–59
Elsevier
BBR 00844

A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data

A. Ennaceur and J. Delacour
Laboratoire de Psychophysiologie, Université Paris VII, Paris (France)

(Received 20 Januari 1988)
(Revised version received 15 March 1988)
(Accepted 16 March 1988)

Key words: Exploration; Memory-recognition; Habituation; Rat

In this paper we describe a new memory test in rats, based on the differential exploration of familiar and new objects. In a first trial (T1), rats are exposed to one or to two identical objects (samples) and in a second trial, to two dissimilar objects, a familiar (the sample) and a new one. For short intertrial intervals (≈ 1 min), most rats discriminate between the two objects in T2: they spend more time in exploring the new object than the familiar one. This test has several interesting characteristics: (1) it is similar to visual recognition tests wideley used in subhuman primates, this allows interspecies comparisons; (2) it is entirely based on the spontaneous behavior of rats and can be considered as a 'pure' working-memory test completely free of reference memory component; (3) it does not involve primary reinforcement such as food or electric shocks, this makes it comparable to memory tests currently used in man.

INTRODUCTION

According to recent advances in clinical neuropsychology, at least two types of memories should be distinguished from the analysis of the global amnestic syndromes: the first is spared, while the second is deeply disturbed. There is no general agreement on the definition of these two kind of memories. Depending on the authors, type I (spared) is the semantic, the reference or the procedural memory, type II (disturbed) is the episodic, the working or the declarative memory[3–5,23,26,31,36,38,40,41,43].

A similar hypothesis was at the basis of an experimental research developed in monkeys years ago by Mishkin and Delacour[25]: a critical factor in the pathology of memory would be the repetition. Memory of a unique event would be much more vulnerable than that based on the repetition of some conditions, such as the association of stimulus or responses to a reinforcer. From this hypothesis, a trial-unique memory test, based on a delayed matching or non-matching procedure, was developed in monkeys[25] and proved to be sensitive to temporal lobe stimulation[8] or lesions[9,26].

However, this test is not completely satisfactory: it is not purely one trial since it is based on the learning of a rule (matching or non-matching to sample) through the repetition of stimulus or response reward associations during a pretraining stage. Alteration of performances may be due to

Correspondence: A. Ennaceur, Laboratoire de Psychophysiologie, Université Paris VII, 7 Quai Saint Bernard, Bât. B, 5° et. 75251 Paris Cedex 05, France.

different causes: either to effects on the one trial component, or on the learning of the rule or on both.

A recent 'rat' version of this task[1,35] suffers from the same drawbacks; in this respect it is not superior to the delayed reinforced alternation, the most popular working memory test in the rat.

The task described in this paper is really a one-trial task. It does not involve at all the learning of a rule since it is entirely based on the spontaneous exploratory behavior of rats towards objects.

MATERIALS AND METHODS

Subjects

A total of 220 male Wistar rats weighing 200–250 g were used. They were housed in individual cages during the testing period. The light–dark cycle was 12: 12 (07.00–19.00 h) and the ambient temperature was 23 ± 1 °C. During the entire experiment they had free access to food and water.

Apparatus

The apparatus used 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. One 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.

EXPERIMENT 1

Subjects

A total of 90 rats that had received only one medium dose of a nootropic drug 10 days before were randomly allocated to 4 groups.

Behavioral testing

Animals were handled and weighed each day. A day before testing they were allowed to explore the box for 2 min. They were then given two testing sessions separated by a 48 h delay. Throughout the experiment no cleaning of the box was allowed, in order to saturate it with olfactory stimuli.

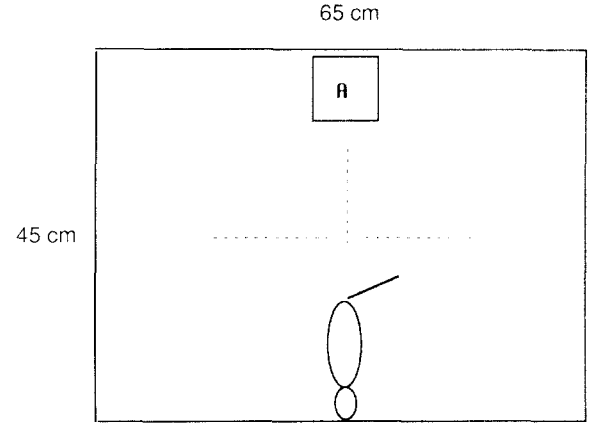

Fig. 1. Representation of experimental conditions in T1, Expt. 1.

Each session was comprised of two trials. In the first trial (T1), one object-stimulus, the sample (A), was placed aside the rear wall of the box in a location equidistant from the back corners of the box (see Fig. 1). During the second trial (T2), a new object (B) was added (see Fig. 5). Here, each object was placed in a back corner. 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) and the position of the two objects during T2 was counterbalanced and randomly permuted. A different pair of objects was used for each session. We shall 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 the opposite. These precautions were taken to reduce object and place preference effects. It should be stressed that the objects apparently had no natural significance for rats and had never been associated to reinforcement.

At the begining of each trial, the rats were placed near the center of the front wall of the box, their heads oriented in the opposite direction to the object. The respective duration of T1 and T2

was 5 and 3 min. Rats for which the intertrial interval was greater than 1 min were taken back to their home cages during this interval.

According to the group, the intertrial delay was: 1 min (G1, $n = 23$); 1 h (G2, $n = 23$); 4 h (G3, $n = 22$); 24 h (G4, $n = 22$).

Measurements and statistical treatments

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

Let (a) be the time spent in exploring the sample during T1, (a′) and (b) the time spent respectively in exploring the sample and the new object during T2. The following variables were considered: e1 = (a), e2 = (a′ + b), h1 = (e1 − e2), d1 = (b − a′) and d2 = (d1/e2).

(e1) and (e2) measure the exploration of both objects during T1 and T2 respectively; (h1) may be considered as an index of habituation of the exploratory behavior and (d1), (d2) as amnesic indexes reflecting the discrimination between the new and the familiar objects.

Within groups statistical tests were applied to variables (h1) and (d1). They were based on the paired Student *t*-test. For instance, significance of (h1) for a given group was tested by comparing the mean value of (e1) and (e2) for that group. Between-groups comparisons were based on ANOVA and post-hoc comparisons according to Winer[42]. The significance threshold was 0.05.

Results

One rat of G2 was excluded from the experiment because of its lack of exploration acitivity.

(1) As indicated in Table I, there were no group differences in the time of exploration of the sample in T1 during each session. So we can consider that the different groups were comparable with respect to exploratory behavior. When rats were re-exposed to the sample and a new object, they did not show a significant difference between groups in the total time spent for the exploration of both objects during T2.

(2) The mean value of (a) was comparable for the two sessions, and for either object O1 and O2 of the same pair used as sample (see Materials and Methods). This means that the 4 objects (two pairs) used in the two sessions elicited comparable exploratory behavior.

(3) All rats, independently of their respective groups, spent in T2 more time in exploring the new object than the sample in each session, as indicated in Table II. The highly significant '*t*' obtained for each group means that for each delay, the new object was distinguished from the sample.

(4) According to the overall ANOVA, the index (d1) which reflects the discrimination between the familiar and the new object (see Materials and Methods) shows only a tendency to significance $F_{3,85} = 2.58$, $P \leq 0.057$.

TABLE I

Mean value (± S.E.M.) of the total exploration time (s) during the first trial (e1) and the second trial (e2)

Groups: G1 = 1 min, G2 = 1 h, G3 = 4 h and G4 = 24 h intertrial delays.

Groups	*G1 (n = 23)*		*G2 (n = 22)*		*G3 (n = 22)*		*G4 (n = 22)*	
	(e1)	*(e2)*	*(e1)*	*(e2)*	*(e1)*	*(e2)*	*(e1)*	*(e2)*
Session 1	31.17	49.43	24.09	37.86	30.23	38.32	30.13	34.32
	± 2.74	± 3.33	± 2.17	± 3.12	± 3.16	± 3.26	± 2.51	± 2.23
Session 2	26.13	41.09	30.95	40.73	34.50	40.36	30	40.54
	± 2.25	± 2.94	± 2.84	± 3.75	± 2.51	± 2.66	± 2.50	± 2.71
Session 1 + 2	57.30	90.52	55.05	78.59	64.73	78.68	60.13	74.86
	± 1.93	± 4.99	± 4.40	± 6.03	± 5.33	± 4.85	± 4.36	± 4.28

TABLE II

Comparisons within each group (paired Student's t-test, two-tailed) of the time (s) spent by rats in exploring the sample (a') and the new object (b) in T2, d1 = (b−a')

Groups: G1 = 1 min, G2 = 1 h, G3 = 4 h and G4 = 24 h intertrial delays.

Groups	*Session 1*			*Session 2*		
	(d1)	*t*	*P≤*	*(d1)*	*t*	*P≤*
G1 (*n* = 23)	10.39 ± 2.37	4.39	0.01	9.78 ± 2.11	4.65	0.01
G2 (*n* = 22)	10.32 ± 1.57	6.56	0.01	9.64 ± 2.45	3.93	0.01
G3 (*n* = 22)	8.41 ± 2.01	4.18	0.01	11.36 ± 2.43	4.67	0.01
G4 (*n* = 22)	4.5 ± 1.72	2.56	0.01	4.91 ± 1.91	2.56	0.01

Whereas Fig. 2 suggests that recognition of the sample was less after a 24 h retention interval than after shorter intervals, Table II indicates that recognition as measured by the variable (d1) was still significant after a 24-h retention interval.

EXPERIMENT 2

Whereas the rats' tendencies to explore a new object proved to be a useful measure on object recognition, the duration of the retention interval from 1 min to 24 h did not have very clear-cut effects. In order to make the test more sensitive to retention duration, we modified the procedure: during T1 the time actually spent in exploring (A) was fixed. Rats remained in the apparatus until they spent the required amount of exploration time.

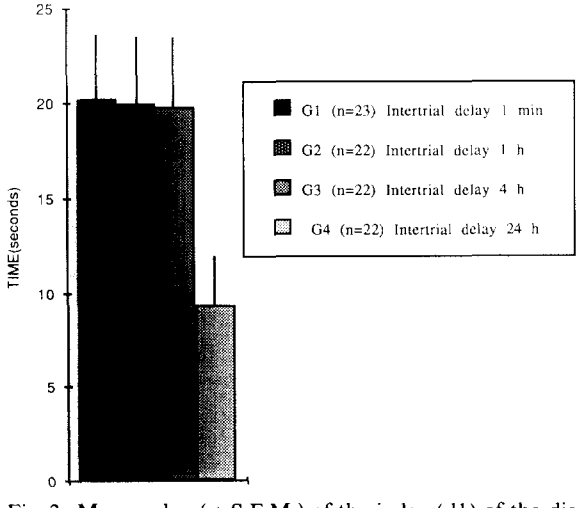

Fig. 2. Mean value (± S.E.M.) of the index (d1) of the discrimination between the new and the familiar objects ($F_{3,85} = 2.58$, $P \leq 0.057$).

Subjects

A total of 40 naive male Wistar rats weighing 200–250 g were used. They were maintained in the same conditions as described previously.

Behavioral testing

As indicated above, during T1, rats had to explore (A) during a fixed duration: 20 s. The trial ended when this exploration criterion was reached. The other conditions and retention intervals were as in Expt. 1: 1 min for G1 (*n* = 10), 1 h for G2 (*n* = 10), 4 h for G3 (*n* = 10) and 24 h for G4 (*n* = 10).

Measurements and statistical treatments

(1) Object exploration was defined as in Expt. 1. We measured the mean duration time in T1 necessary for a 20-s exploration of (A). We also measured the time spent by rats in exploring the two objects in T2.

(2) Other variables were as in Expt. 1.

(3) Student's *t*-test and one-way analyses of variance were performed.

TABLE III

Mean (± S.E.M.) time needed by rats to make 20 s exploration of the sample in the first trial (e1) and the total exploration time (s) of the two objects in the second trial (e2)

Groups: G1 = 1 min, G2 = 1 h, G3 = 4 h and G4 = 24 h intertrial delays.

Groups	*G1 (n = 9)*		*G2 (n = 10)*		*G3 (n = 10)*		*G4 (n = 9)*	
	(e1)	*(e2)*	*(e1)*	*(e2)*	*(e1)*	*(e2)*	*(e1)*	*(e2)*
Session 1	172.67 ± 23.41	48.33 ± 4.94	177.30 ± 16.25	38.40 ± 6.23	152.70 ± 17.81	46.50 ± 3.63	168.44 ± 20.26	40.22 ± 3.19
Session 2	129.44 ± 14.24	38.78 ± 5.98	110.70 ± 24.64	42 ± 3.32	94.50 ± 23.0	31.8 ± 4.68	135.33 ± 29.48	46 ± 4.64
Session 1 + 2	302.11 ± 27.1	87.11 ± 9.59	288 ± 30.58	80.4 ± 7.49	247.2 ± 32.86	78.3 ± 7.11	303.78 ± 35.87	86.22 ± 4.45

Results

Two rats were excluded from the experiment because of their lack of exploration, one of G1 and one of G4.

(1) The mean time needed by rats, during T1 for a 20-s exploration of the sample (A) and the total time of exploration during T2 are shown in Table III. Overall analyses of variance did not indicate any differences between groups for each session.

(2) Discrimination between the new and the familiar object was significant only in G1 for a 1-min retention interval (paired Student's *t*-test).

(3) The overall analyses of variance of measures of the indexes (d1) and (d2) show only tendencies to significance between groups, $F_{3.34} = 2.45$, $P \leq 0.08$ and $F_{3.34} = 2.42$, $P \leq 0.08$, respectively.

On the contrary to Expt. 1, only G1 (1-min delay) showed discrimination between the familiar and the new objects. This discrepancy between the two experiments is probably due to the difference in the duration of the exploration of the sample during T1 (see Table I): the fixed duration, 20 s, allowed to rats in Expt. 2 is significantly inferior to that spent by rats in Expt. 1 in 5 min according to Student's *t*-test ($P \leq 0.01$).

TABLE IV

Comparisons within each group (paired Student's t-test, two-tailed) of the time spent by rats in exploring the sample (a') and the new object (b) in T2, d1 = (b–a')

Groups: G1 = 1 min, G2 = 1 h, G3 = 4 h and G4 = 24 h intertrial delays.

Groups	*Session 1*			*Session 2*		
	(d1)	*t*	*P≤*	*(d1)*	*t*	*P≤*
G1 (*n* = 9)	12.55 ± 3.55	3.72	0.01	8.78 ± 4.32	2.03	0.05
G2 (*n* = 10)	2.4 ± 2.12	1.13	n.s.	6 ± 4.04	1.48	n.s.
G3 (*n* = 10)	4.1 ± 2.82	1.45	n.s.	1.6 ± 1.90	0.84	n.s.
G4 (*n* = 9)	4 ± 3.48	1.14	n.s.	1.11 ± 1.84	0.60	n.s.

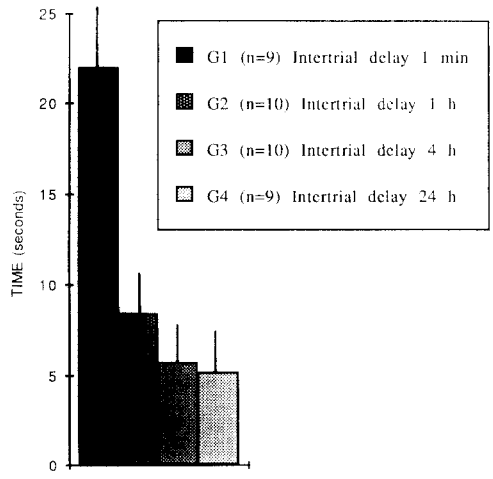

Fig. 3. Mean value (± S.E.M.) of the index (d1) of the discrimination between the new and the familiar objects ($F_{3,34} = 2.61$, $P \leq 0.06$).

EXPERIMENT 3

In this experiment, during T1, two samples were presented instead of one. These samples were identical. The purpose of this procedure was to make T1 and T2 more comparable; in each trial, rats were exposed to two objects. This allowed a more precise evaluation of the habituation occurring from T1 to T2 and a more precise control of place preference.

Subjects

A total of 24 naive male Wistar rats weighing 200–250 g were used. They were maintained in the same conditions as described previously.

Behavioural testing

The basic experimental conditions were similar to those used in Expt. 1, except that two identical objects were used as samples during T1 (see Fig. 4). We shall call them A1 and A2. In T1 they were placed in the back corners of the box. In T2, we replaced one of the samples by a new object (B). As previously, the position of the sample and the new object was randomly permuted from rat to rat and from session to session. Rats were allowed 3 min of exploration for each trial (instead of 5 min for T1 in Expt. 1). The animals were tested in two sessions separated by 48 h. Two

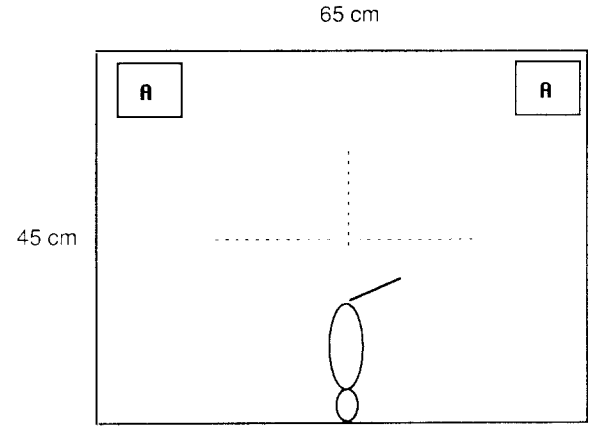

Fig. 4. Representation of the experimental conditions in T1, Expt. 3.

intertrial intervals were used: 1 min for the group 1 (G1, $n = 11$) and 1 h for the group 2 (G2, $n = 13$).

Measurements and statistical treatments

Basic measurements were identical to those of Expt. 1, except that the variable (e1) was constituted by (a1) + (a2): the time spent in exploring each sample during T1. Two other mnesic indexes were added: h2 = [(e1/2) − (a′)] and d3 = (h2) + (d1). The first index (h2) measures differences between the average time spent in exploring the samples in T1 (e1/2) and in T2 (a′). The second index (d3) represent the addition of the two types of discrimination (h2) and (d1).

Statistical analyses were performed on the following measurements:

(1) Similarity of the groups with respect to

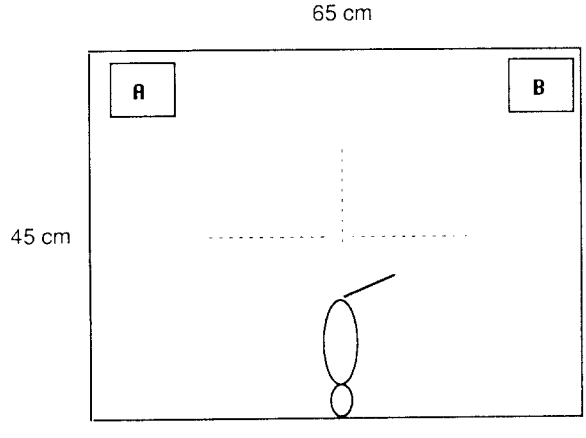

Fig. 5. Representation of experimental conditions in T2.

TABLE V

Mean value (± S.E.M.) of the total exploration time (s) during the first trial (e1) and the second trial (e2)

Comparisons within groups of the total time spent by rats in exploring the objects in T1 and in T2 (paired Student's *t*-test, two-tailed). Groups: G1 = 1 min, G2 = 1 h intertrial delays.

	Session 1				*Session 2*				*Sessions 1 + 2*	
	(e1)	*(e2)*	*t*	*P≤*	*(e1)*	*(e2)*	*t*	*P≤*	*(e1)*	*(e2)*
G1	21.36	17.18	2.24	0.5	18.09	14.00	1.57	n.s.	39.45	31.18
(*n* = 11)	± 2.22	± 2.58			± 2.57	± 2.36			± 4.16	± 4.39
G2	20.61	15.00	2.45	0.5	12.84	11.69	0.47	n.s.	33.46	26.69
(*n* = 13)	± 1.92	± 2.62			± 2.65	± 1.95			± 3.54	± 3.82

exploration was checked by comparing the total time of exploration of samples (e1). Moreover the differences between groups for the exploration of the two objects in T2 (e2) was checked and the index of habituation (h1) was computed.

(2) Side preferences were tested by comparing (a1) and (a2) within each group (paired Student *t*-test).

(3) Two memory-indexes were considered for within group tests: (h2) as an index of habituation to the familiar object and (d1) as an index of discrimination between the familiar and the new object (paired Student *t*-test).

(4) For assessing intertrial delay effect, G1 and G2 were compared by the following index of memory: (h2), (d1), (d2) and (d3).

Results

(1) The overall level of exploration, as evaluated by the index (e1) and (e2), was similar in the two groups.

The index of global habituation to the situation (h1) shows that in both groups the total time spent by rats in exploring objects decreased from T1 to T2 in session 1 but not in session 2. From session to session, there was no significant decrease of the time of exploration in T1 and in T2 for G1, but for

TABLE VI

Comparisons within each group (paired Student's t-test, two-tailed) of the average time (e1/2) spent in exploring the samples in T1 to time spent in exploring the sample in T2: h1 = [(e1/2)–(a')] and comparison within each group of the time spent by rats in exploring the sample (a') and the new object (b) in T2: d1 = (b–a')**.*

*	*Session 1*			*Session 2*		
	(h2)	*t*	*P≤*	*(h2)*	*t*	*P≤*
G1 (*n* = 11)	4.23 ± 1.28	3.3	0.01	6.04 ± 1.47	4.11	0.01
G2 (*n* = 13)	4.23 ± 0.98	4.29	0.01	2.27 ± 1.04	2.17	0.05
**	*(d1)*	*t*	*P≤*	*(d1)*	*t*	*P≤*
G1 (*n* = 11)	4.27 ± 1.59	2.68	0.02	8 ± 1.71	4.66	0.01
G2 (*n* = 13)	2.84 ± 1.09	2.61	0.02	3.38 ± 1.62	2.09	0.05

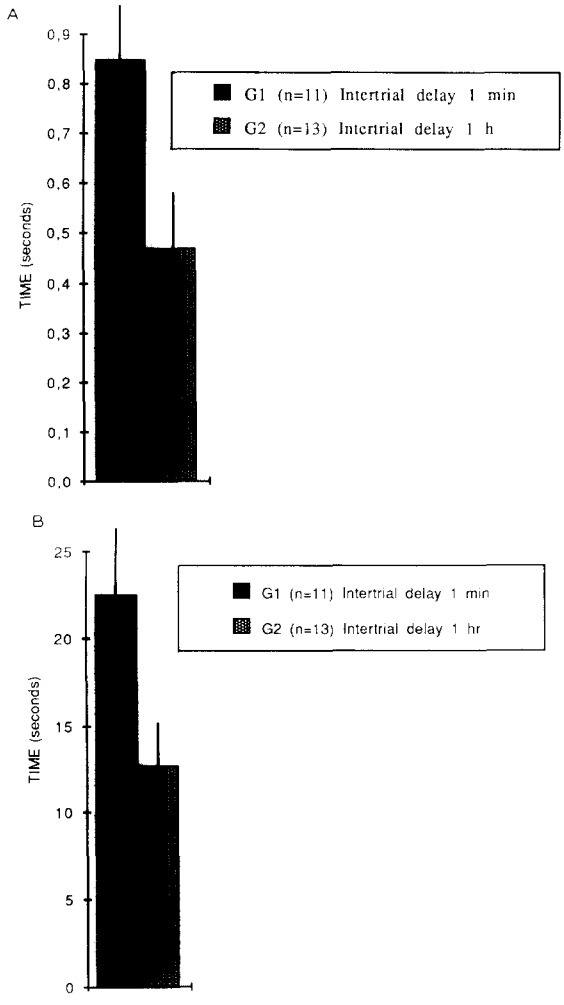

Fig. 6. A. Mean value (± S.E.M.) of the index of discrimination (d2) which is constituted by the index of discrimination (d1) divided by the index of overall exploratory activity in T2 (e2) ($F_{1,22} = 4.18$, $P \leq 0.05$). B. mean value (± S.E.M.) of the index (d3), which combines the index for habituation to the sample (h2) and the index of discrimination between the new and the familiar objects (d2) ($F_{1,22} = 4.60$, $P \leq 0.03$).

G2 the time spent in session 2 in exploring the samples in T1 was significantly inferior to the time spent by the same group in session 1.

(2) No significant side preference was detected within each group. The time spent in exploring each sample was comparable.

(3) The two indexes of memory (h2) and (d1) are significant within both groups (paired Student *t*-test), which means that the sample was less explored during T2 than during T1, and was less explored than the new object in T2.

(4) Comparisons between groups showed that the two indexes of memory (d2) and (d3) were significantly less in G2 (1 h delay) as compared to G1 (1 min delay).

EXPERIMENT 4

The purpose of this experiment was to precise the influence of the retention time (the delay between T1 and T2) on the performances of rats in the new version of the test presented in experiment 3.

Subjects

A total of 20 naive male Wistar rats weighing 200–250 g were used. They were maintained in the same conditions as described previously.

Behavioural testing

Experimental conditions and behavioural testing were similar to those used in Expt. 3; however all the rats were submitted in a random sequence to 3 different intertrial delays (D1 = 1 min, D2 = 1 h and D3 = 24 h), one session per delay. The intersession interval was 48 h.

Measurements and statistical treatments

Basic measurements were identical to those of Expt. 3. The data were submitted to one-factor ANOVA for repeated measures and to the Student *t*-test when appropriate.

Results

Two rats were excluded from the experiment because of their lack of exploration activity.

(1) There was no significant object and side preferences for any delay.

(2) The overall levels of exploration of objects in T1 and in T2 were not significantly different, and there were no significant differences in the global index of habituation (h1) according to the delay.

(3) The rats discriminated between the familiar and the new object when the intertrial delay was of 1 min or 1 h, but not after a 24-h delay.

(4) The length of the intertrial delay had an effect on the index of discrimination (d3), it is

TABLE VII

Mean value (± S.E.M.) of the total exploration time (s) during the first trial (e1) and the second trial (e2) and comparisons between these values which are an index (h1) of the global habituation (paired Student's t-test)

Groups: D1, D2, D3 = 1 min, 1 h and 24 h intertrial delay respectively.

	D1	*D2*	*D3*
(e1)	18.39 ± 1.84	17.22 ± 2.05	16.17 ± 1.47
(e2)	17.78 ± 2.53	18.22 ± 1.86	17.44 ± 2.06
t	0.22	0.42	0.61
$P \leq$	n.s.	n.s.	n.s.

significantly inferior when the delay is of 24 h than when it is of 1 min.

EXPERIMENT 5

In the previous experiments, we have demonstrated that object-recognition can be measured by the time spent by rats in exploring familiar and new objects. Most rats spent more time in exploring the new object. Recognition performance varies according to the delay between T1 (presentation of object sample) and T2 (presentation of the sample and the new objects), and to the time allowed to rats in exploring the sample in T1. The purpose of this experiment was to control these results in exposing rats to a pair of familiar objects or to a pair of new objects in T2 after a standard T1.

TABLE VIII

Comparison within each group (paired Student's t-test, two-tailed) of the average time (e1/2) spent by rats in exploring the samples in T1 to time spent for the sample (a') in T2: h2 = [(e1/2)–(a')] and comparisons within each group of the time spent by rats in exploring the sample (a') and the new object (b) in T2: d1 = (b–a')***

Groups: D1, D2, D3 = 1 min, 1 h and 24 h intertrial delay respectively.

	*(h2)**	*t*	$P \leq$	*(d1)***	*t*	$P \leq$
D1	3.14 ± 1.32	2.38	0.025	5.67 ± 1.90	2.97	0.01
D2	1.11 ± 1.33	0.83	n.s.	3.22 ± 1.08	2.96	0.01
D3	0.25 ± 1.16	0.21	n.s.	1.78 ± 0.88	2	n.s.

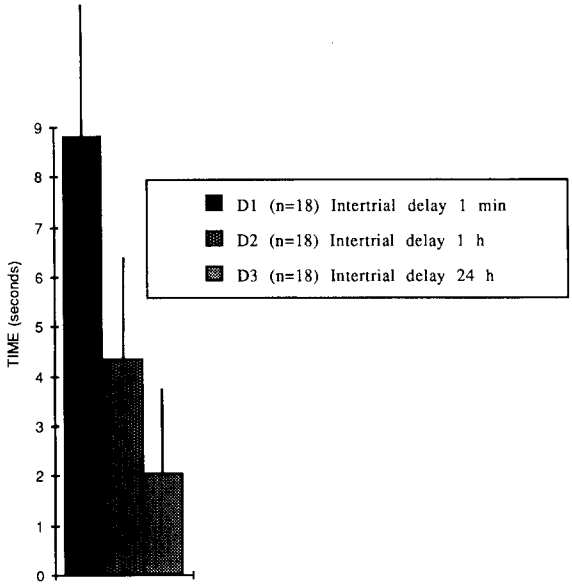

Fig. 7. Mean value (± S.E.M.) of the index (d3), which combined the index for habituation to the sample (h2) and the index of discrimination between the new and the familiar objects (d2) ($F_{2,34} = 3.39$, $P \leq 0.05$).

Subjects

A total of 46 naive male Wistar rats weighing 200–250 g were used. They were maintained in the same conditions as in the previous experiments.

Behavioral testing

The rats were handled and exposed to experimental conditions for a 2-min session by day for 2 days. Testing began the third day for only one session. It was conducted as described in Expt. 1; however, according to the groups, the objects presented in T2 could be AA (which is a pair of objects identical to the sample), BB (a pair of two identical new objects) or AB (the sample and a new object).

The duration of T1 and T2 was 3 min and the intertrial delay 1 min.

The 3 groups will be termed according to the kind of stimuli presented in T2: AA ($n = 15$),

BB (n = 16) and AB (n = 15). The animals were tested in a random order.

Measurements and statistical treatments

Several variables were as in Expt. 1.

(1) Level of exploration was measured by (e1) and (e2), respectively the time spent in exploring the sample in T1 and the time spent in exploring the two objects in T2.

(2) Object preferences were tested by comparing the time (a) for the two different types of samples used with different subgroups of rats.

(3) Side preferences were measured in groups AA and BB by the variables (a1–a2) and (b1–b2) in T2.

(4) Recognition was measured within group AB by the variables (b–a'). This measure was compared to the variables (a1–a2) and (b1–b2) in groups AA and BB respectively. In these groups, the time spent in exploring the object placed in the left corner, was arbitrarily termed a1 (respectively b1) and that in the right corner a2 (respectively b2).

Results

(1) As shown in Table IX, there are no differences between groups in the exploration of the sample in T1, $F_{2,43} = 1.93$, $P > 0.10$.

(2) No significant differences were observed in the exploration of either objects presented as samples in T1, $F_{1,44} = 0.002$, $P > 0.10$.

TABLE IX

Mean (± S.E.M.) time (s) exploration of the sample in T1 (e1) and of the two objects in T2 (e2)

Groups	*AA*	*BB*	*AB*
(e1)	19.47	16.69	21
	± 1.78	± 1.23	± 1.56
(e2)	24.47	22.25	23.40
	± 2.17	± 1.49	± 2.21

(3) There were no significant differences in the total time spent by the different groups in exploring both objects in T2 (see Table IX), which means that the level of arousal, 'curiosity', was comparable in the 3 groups.

(4) Discrimination between objects during T2 was observed only in group AB where the sample was presented simultaneously with a new object: according to within-group tests, group AB spent more time in exploring the new object than the sample in T2. On the contrary, in the AA or BB groups, time spent in exploring either objects was comparable. Moreover, comparisons between groups showed that the discrimination index (b–a') in group AB was significantly higher than (a1–a2) in group AA and (b1–b2) in group BB.

This experiment shows that object recognition is reflected by a systematic factor such as the difference in exploration time of the new and the familiar objects which is significantly different from differences in exploration of two identical objects, either familiar or new.

TABLE X

Comparisons between groups of the mean value (± S.E.M.) of the variable (b–a') in group AB, (a1–a2) in group AA and (b1–b2) in group BB and comparisons within each group (paired Student's t-test, two-tailed) of the time spent in exploring the new and the familiar objects for group AB, two new identical objects for group BB or two familiar ones for group AA**.*

Groups	*AA*	*BB*	*AB*	*Overall analyses of variance*		
Variables	*(a1–a2)*	*(b1–b2)*	*(b–a')*	$F_{2,43}$	$P \leq$	
	0.33	− 0.375	5.40	± 12.18	0.01	*
	± 0.66	± 0.76	± 1.18			
t	0.55	0.49	4.58			**
$P \leq$	n.s.	n.s.	0.01			

DISCUSSION

The main results of these experiments may be summarized as follows.

(1) Object-recognition in rats may be measured by the difference in the exploration time of new and familiar objects. Rats spend more time in exploring the new objects. This preference is observed in almost all normal rats; it is well above the level of random variations of the exploration behavior.

(2) This recognition measure is influenced by the interval between T1 and T2 as well as the time allowed for rats to explore the sample in T1.

(3) A wider range of variables that can be sensitive to brain lesions and pharmacological treatments (in the following paper) are available (See preliminary study: 11). In its last version, our test gives:

1. *Two main indexes of recognition*

(a) Habituation to the sample: it is measured by comparing the average time spent in exploring the samples in T1 to the time spent in exploring the sample in T2.

(b) Discrimination between the new and familiar objects: it is measured in T2 by comparing the time spent in exploring the sample to that spent in exploring the new object.

The measure of these two indexes are not independent; however, they cannot be considered as equivalent. They were combined as two estimations of the recognition process.

2. *An index of global habituation:* it is measured by comparing the total time spent in exploring the two objects in T1 to that spent in T2.

3. *Control measures of:*

(a) The overall level of exploration.

(b) The side and object preferences.

The spontaneous tendency – preference of novelty – on which our test is based is present in primates as well as in rodents[21,27,28,32,33], (for review see ref. 6) and was studied in different ways by many authors[2,10,12–14,24,29,30,34]. However, unlike the works of Dember[10] and Gaffan[13] in our test, (1) the rats experienced the two objects presented in an acquisition trial, (2) the discrimination between familiar and new objects is measured by the time spent for each object and not on the basis of the first choice, (3) the 'mnemonic' materials are objects instead of the visual aspect of a place.

The preference of novelty as described in our experiments gives a large field of application to our test: it measures memory in rats according to a protocol similar to that used in subhuman primates and this allows comparisons between species (see refs. 7, 15, 37, 39, 44). However, as already stressed in the Introduction, our test differs from similar tasks[1,2,13,15–17,25,35] in two respects: (1) it has no 'reference memory' component such as rule learning: the choice of the arm previously closed which is the feature of the delayed alternation test. In this respect our test can be considered as a 'pure' working memory test, (2) it is not based on usual positive or negative reinforcers such as food or electric shocks which make the interpretation of the effects of brain modification on memory difficult[18–20,22]. The strong involvement of these reinforcements in most tasks used with animals is probably one of the causes of difficulty in reproducing experimentally the amnesic syndromes, since human learning and memory capacities are not usually tested under high fear or hunger levels.

Due to these characteristics, our test may be a useful tool for pharmacological and neurological studies on the memory of rats. The results of the experiments described in the companion paper seem to confirm this prospect.

ACKNOWLEDGEMENTS

We thank Mrs. S. Walker for the editing of this paper. This research was supported by D.R.E.T. Grant 86/055.

REFERENCES

1 Aggleton, J.P., One-trial object-recognition by rats, *Q. J. Exp. Psychol.*, 37B (1985) 279–294.

2 Alexinsky, T. and Chapoutier, G., A new behavioral model for studying delayed response in rats, *Behav. Biol.*, 24 (1978) 442–456.

3 Baddely, A., Amnesia: a minimal model and an interpretation. In L.S. Cermak (Ed.), *Human Memory and Amnesia*, Lawrence Erlbaum, Hillsdale, NJ, 1982, pp. 305–336.

4 Cermak, L.S., The episodic/semantic distinction in amnesia. In N. Butters and L.R. Squire (Eds.), *The Neuropsychology of Memory*, Guillord, New York, 1984.

5 Cohen, N.J., Preserved learning capacity in amnesia: evidence for multiple memory systems. In N. Butters and L.R. Squire (Eds.), *The Neuropsychology of Memory*, Guilford, New York, 1984.

6 Corey, D.T., The determinants of exploration and neophobia, *Neurosci. Biobehav. Rev. 2*, (1978) 235–253.

7 Delacour, J. and Borst, A., Failure to find homology in rat, cat and monley for functions of a subcortical structure in avoidance conditioning, *J. Comp. Physiol. Psychol.*, 80 (1972) 458–468.

8 Delacour, J., Role of temporal lobe structures in visual short-term memory, using a new test, *Neuropsychologia*, 15 (1977) 681–684.

9 Delacour, J., Cortex inferotemporal et mémoire visuelle à court terme chez le singe. Nouvelles données. *Exp. Brain Res.*, 28 (1977) 301–310.

10 Dember, W.N., Response by rats to environmental change, *J. Comp. Physiol. Psychol.*, 49 (1956) 93–95.

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

12 File, S.E. and Wardill, A.G., Validity of head-dipping as a measure of exploration in a modified hole-board, *Psychopharmacologia*, 44: 53–59.

13 Gaffan, D., Loss of recognition memory in rats with lesions of the fornix, *Neuropsychologia*, 10 (1972) 327–341.

14 Gaffan, E.A. and Davies, J., Reward, novelty and spontaneous alternation, *Q. J. Exp. Psychol.*, 34B (1982) 31–47.

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

16 Gaffan, D., Gaffan, E.A. and Harrison, S., Effects of fornix transection on spontaneous and trained nonmatching by monkeys, *Q. J. Exp. Psychol.*, 36B (1984) 285–303.

17 Gaffan, D., Shields, C. and Harrison, S., Delayed matching by fornix transected monkeys: the sample, the push and the bait, *Q. J. Exp. Psychol.*, 36B (1984) 305–317.

18 Gaffan, D., Saunders, R.C. and Gaffan, E.A., Effect of fornix transection upon associative memory in monkeys: role of the hippocampus in learned action, *Q. J. Exp. Psychol.*, 36B (1984) 173–221.

19 Huston, J.P., Mondadori, C. and Waser, P.G., Facilitation of learning by reward post-trial memory processes, *Experientia*, 30 (1974) 1038–1040.

20 Jaffard, R., Dubois, M. and Galey, D., Memory of a choice direction in a T-maze as measured by spontaneous alternation in mice: effects of intertrial interval and reward, *Behav. Proc.*, 6 (1981) 11–21.

21 Joubert, A. and Vauclair, J., Reaction to novel objects in a troop of guinea baboons: approach and manipulations, *Behaviour*, 96 (1986) 92–104.

22 Ketty, S.S., Biological concomitants of affective states and their possible role in memory processes. In M.R. Rosenzweig and E.L. Bennett (Eds.), *Neural Mechanisms of Learning and Memory*, MIT Press, 1976, pp. 321–326.

23 Kinsbourne, M. and Wood, F., Short-term memory and the amnesic syndrome. In D.D. Deutsch and J.A. Deutsch (Eds.), *Short-term Memory*, Academic, New York, 1975.

24 Lukaszewska, I. and Mlodkowska, Z., Exploratory motivation in response to change tests in rats, *Acta Neurobiol. Exp.*, 40 (1980) 161–171.

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

26 Mishkin, M. and Petri, H.L., Memories and habits: some implications for the analysis of learning and retention. In L.R. Squire and N. Butters (Eds.), *Neuropsychology of Memory*, Guilford, New York, 1984, pp. 287–296.

27 Mislin, R. and Ropartz, R., Response in mice to a novel object, *Behaviour*, 78 (1981) 169–177.

28 O'Keefe, J. and Nadel, L., *The Hippocampus as a Cognitive Map*, Clarendon, Oxford, 1978, pp. 240–264.

29 Olton, D.S., Becker, J.T. and Handelmann, G.E., Hippocampal function: working memory or cognitive mapping? *Physiol. Psychol.*, 8 (1980) 239–246.

30 Olton, D.S., Becker, J.T. and Handelmann, G.E. Hippocampus, space and memory, *Behav. Brain Sci.*, 2 (1979) 313–365.

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

32 Renner, M.J., Experience-dependent changes in exploratory behavior in the adult rat (*Rattus Norvegicus*): overall activity level and interactions with objects, *J. Comp. Psychol.*, 101 (1986) 94–100.

33 Renner, M.J. and Rosenzweig, M.R., Object interactions in juvenile rats (*Rattus Norvegicus*): effects of different experiential histories, *J. Comp. Psychol.*, 100 (1986) 229–236.

34 Roberts, W.W., Dember, W.N. and Brodwick, M., Alternation and exploration in rats with hippocampal lesions, *J. Comp. Physiol Psychol.*, 55 (1962) 695–700.

35 Rothblat, L.A. and Hayes, L.L., Short-term object-recognition memory in the rat: non-matching with trial unique junk stimuli, *Behav. Neurosci.*, 101 (1987) 587–590.

36 Shachter, D.L., Multiple forms of memory in humans and animals. In N.M. Weinberger, J.L. McGauch and G. Lynch (Eds.), *Memory Systems of the Brain*, Guilford, New York, 1985, pp. 351–379.

37 Squire, L.R. and Zola-Morgan, S., The neuropsychology of memory: new link between human and experimental animals, *Ann. N.Y. Acad. Sci.*, 444 (1985) 137–149.

38 Squire, L.R. and Cohen, N.J., Human memory and amnesia. In G. Lynck, J.L. McGaugh and N.M. Weinberger (Eds.), *Neurobiology of Learning and Memory*, Guilford, New York, 1984.

39 Squire, L.R., Zola-Morgan, S., The neurology of memory: the case for correspondance between the findings for

human and non-human primates. In J.A. Deutsch (Ed.), *The Physiological Basis of Memory*, Academic New York, 1983, pp. 199–268.

40 Tulving, E., Precis of element of episodic memory, *Behav. Brain Sci.*, 7 (1984) 223–268.

41 Weiskrantz, L., Comparative aspects of studies in amnesia, *Phil. Trans. R. Soc. Lond. Ser. B.*, 298 (1982) 97–109.

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

43 Wood, F., Ebert, V. and Kinsbourne, M., The episodic-semantic memory distinction in memory and amnesia: clinical and experimental observation. In L.S. Cermak (Ed.), *Human Memory and Amnesia*, Lawrence Erlbaum, Hillsdale, New Jersey, 1982.

44 Zola-Morgan, S. and Squire L.R., Medial temporal lesions in monkey's impair memory on a variety of tasks sensitive to human amnesia, *Behav. Neurosci.*, 99 (1985) 22–34.