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The double Y-maze as a tool for assessing memory in rats

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1. DESCRIPTION

In many tasks used to evaluate memory in rats, it is difficult to attribute a deficit unequivocally to a dysfunction of memory since deficits may be attributed equally to non-mnemonic variables including motor capacity, perception and motivation[9]. One way to overcome this difficulty is to devise a task that has distinct reference (trial-independent) memory[10] and working (trial-dependent) memory[10] components that each make equal demands on non-mnemonic variables. In such a task, observation of a specific working memory deficit following some treatment would strongly suggest that the treatment affected memory.

The double Y-maze is a task in which reference memory and working memory are dissociable. Assessment of reference memory, which takes place in the first half of the maze, is conducted with the use of a task which remains constant over trials. Rats are randomly placed in one of two arms and must traverse the stem for food reward. Assessment of working memory, which takes place in the second half of the maze, is conducted with the use of a task which requires recall of the starting position from the first half of the maze. Rats must remember which arm they were placed in during the reference memory task in order to correctly choose between the two arms in the working memory task. Since both tasks are identical except for the type of memory required for accurate performance, the differential effects of experimental manipulations (e.g. drugs, excitotoxic lesions) on working and reference memory can be surveyed. Moreover, unlike some tasks (e.g. the radial maze), the probability of making either a reference or a working memory error is equal for both the reference and working memory components; thus, a direct comparison between the two is possible.

2. TYPE OF RESEARCH

1. Development of animal models of memory impairment.

2. Evaluating variables which might affect memory (e.g. aging, diet).

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3. Evaluating the efficacy of neural grafts for the treatment of mnemonic deficits.

4. Screening of potential pharmacological compounds for the treatment of mnemonic deficits.

3. MATERIALS

Each arm of the double Y-maze (Fig. 1) is 35 cm long and extends from the central stem (45 cm long and 17 cm wide) at a 120° angle. The floor is constructed of parallel 17 cm long stainless steel bars, spaced approximately 0.9 cm apart, with the exception of the junctions where the arms meet the stem, which are constructed of 17 cm equilateral clear plastic triangles. The walls of the maze are constructed of 1 cm thick plywood. Removable wooden doors are used to permit or restrict entry into each half of the maze. The doors and maze walls (26 cm high) are painted flat grey. Small pieces of Froot Loops cereal (Kellogg's) are used as reward, and are also scattered beneath the floor to prevent the animals from smelling which arm contains food. The central stem, as well as the distal end of each arm, each contain a metal food cup (4 cm wide and 1 cm deep). The entire maze is supported on a table (70 cm above floor level) in a room where various visual cues can be readily viewed by the rat in the maze (e.g. door, light fixture).

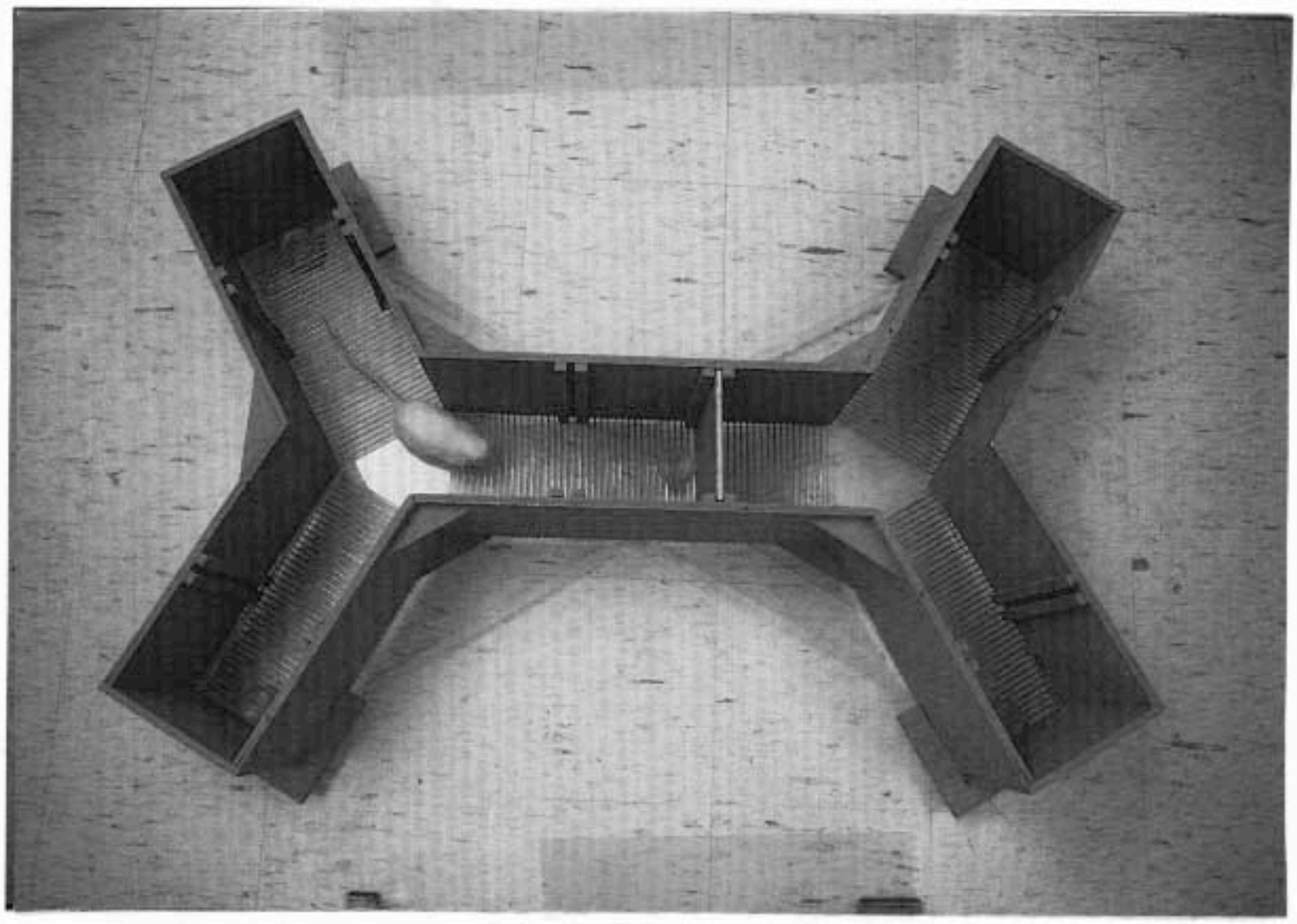

Fig. 1. Photograph of the double Y-maze.

4. DETAILED PROCEDURE

Pre-training

A. Rats (200–250 g) should be housed in a temperature-controlled room in which the lights are on for 12 h each day, and handled for at least 5 min daily. If food deprivation is to be used, reduce the daily ration of food for one week until animals reach the desired body weights (85% of free-feeding weight is typical). Weights should be adjusted for growth (add approximately 5 g per week) during the course of the experiment.

B. Place a small quantity of the Froot Loops cereal in the home cages a few days prior to the commencement of the study. This will reduce the time required to habituate the animals to the maze since rats will not readily eat a novel food when first exposed to the maze.

C. Remove all maze doors, place several small pieces of Froot Loops cereal in food cups A′, B′ and C (Fig. 2), and allow the animals to explore the entire maze and collect food for 15 min per day for at least 3 days. Do not place food in cups A and B during habituation trials since the lack of food in these arms should result in a decreased tendency to choose these arms, and therefore expedite the training process.

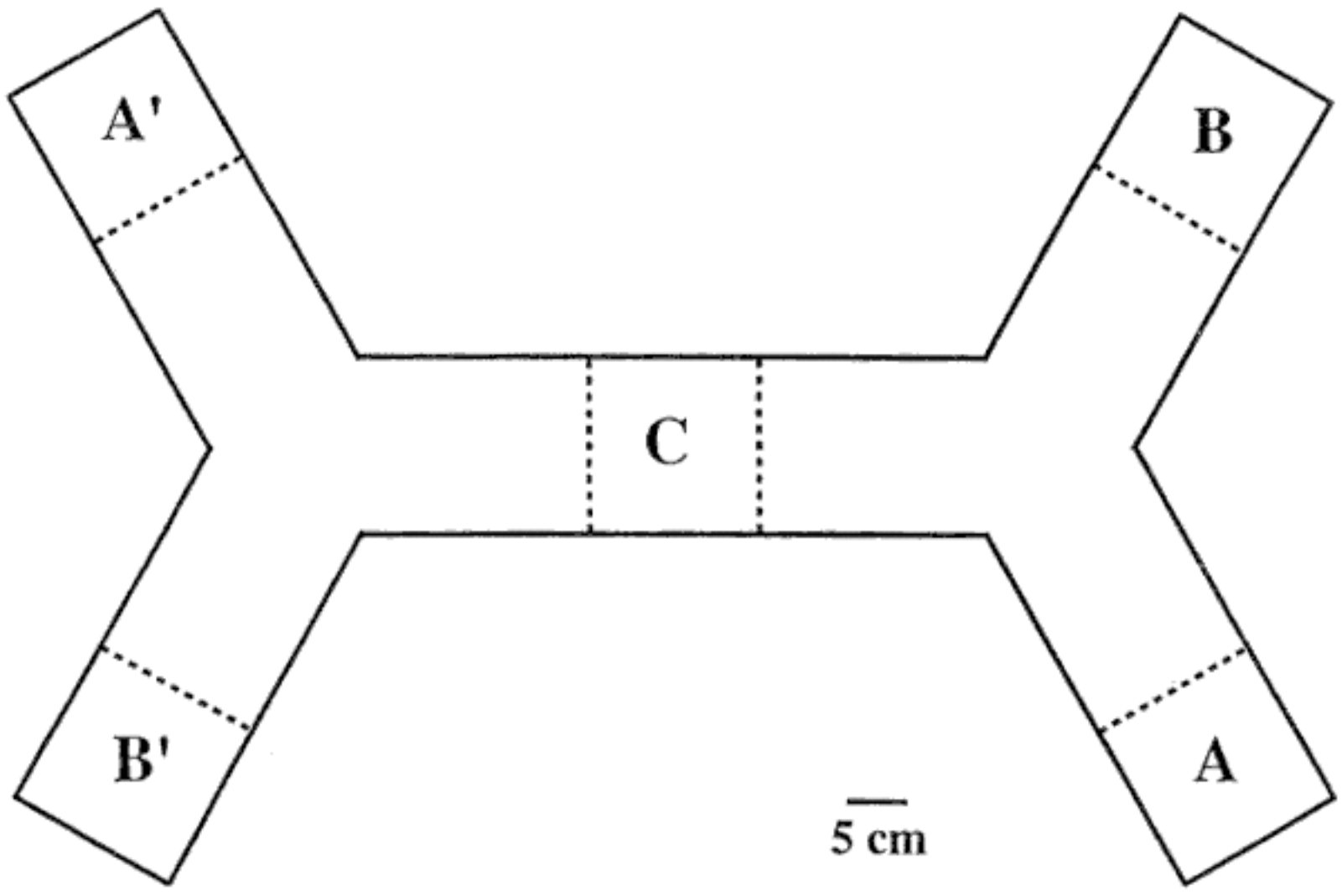

Fig. 2. Top view of the double Y-maze. If a trial begins in A, the correct reference memory choice is C, and the correct working memory choice is A′. If a trial begins in B, the correct reference memory choice is C, and the correct working memory choice is B′. Dashed lines represent the placement of removable wooden doors.

Training/Testing

D. Place a small piece of Froot Loops cereal in the central food cup (C) and in one of the goal boxes in the second half of the maze (either A′ or B′).

E. Insert a door on the far side of box C and at either box A or B, whichever one will serve as the start position on this trial.

F. Place the rat in the box which is blocked by a door. If the rat is placed in box A, then the correct reference memory choice is C and the correct working memory choice is A′. If the rat is placed in box B, then the correct reference memory choice is also C, but the correct working memory choice is B′. Although this configuration can be reversed for some subjects within a study so that trials beginning in box A are rewarded in boxes C and B′, and trials beginning in box B are rewarded in boxes C and A′ for some animals, observations from our laboratory have shown that these two configurations do not have similar acquisition rates (the latter requires a greater number of trials to acquisition).

G. If the animal proceeds to box C, place a door behind the animal so that access to the first half of the maze now is restricted, and remove the door in front of the animal so that access to the second half of the maze now is allowed. This constitutes a correct reference memory choice. If, on the other hand, the animal does not proceed to box C, but chooses the other start box (A or B), remove the animal from the maze and score the trial as a reference memory error.

H. Remove the rat when the second goal box is reached (A′ or B′) and the reward is consumed. If a working memory error is committed, allow the rat to proceed to the correct arm and eat the food reward before being removed from the maze (correction trial). Do this only for the first two or three hundred training trials. Remove the rat from the maze following a working memory error on subsequent training trials. Although data have not been systematically collected, observations from this laboratory have found this to be the most effective training strategy.

I. Repeat steps D to H for the desired number of trials which constitute a session until criterion is reached. Typically, we have used 30–50 trials/session, one session per day, and a training criterion of 80–90% correct for both the reference and working memory components over 3 consecutive days. If time is limited, the number of trials can be reduced to 12–24 trials per session once rats are approaching criterion. However, the number of trials per session should be held constant for the testing phase of an experiment since an unequal number of trials would give some animals more post-treatment practice than others and could bias the comparison between subjects.

5. RESULTS

Table I shows the effects of various experimental manipulations on the reference and working memory components of the double Y-maze. Selective working memory deficits were observed following bilateral quisqualic acid lesions of the nucleus basalis magnocellularis (NBM)[3,6], unilateral phthalic acid lesions of the NBM[12], systemic scopolamine (0.4 mg/kg)[5], bilateral intra-NBM muscimol infusions (0.1

μg in 0.5 μl)[2], bilateral intra-amygdala infusions of scopolamine (24 μg in 0.5 μl)[11] and aging (8 vs. 16 months)[5]. Reference and working memory both were affected by bilateral ibotenic acid lesions of the NBM[3], a higher systemic dose of scopolamine (0.8 mg/kg)[5], a higher intra-NBM dose of muscimol (1.0 μg in 0.5 μl)[2], and a higher intra-amygdala dose of scopolamine (72 μg in 0.5 μl)[11]. In addition, results taken from Biggan and associates[6] which utilized delays (0, 5 and 30 s) placed before both the reference and the working memory components of the double Y-maze task are presented in Fig. 3. A delay-dependent decrease in the working memory component but no effect in the reference memory component was found. These results are in accord with previous studies showing that working memory is susceptible to delay-dependent interference[14–16].

TABLE I: Observed mnemonic deficits in double Y-maze investigations

Independent variable	Working memory	Reference memory	Reference
Biliateral quisqualic acid nucleus basalis magnocellularis (NBM) lesions	↓	–	Biggan, Beninger, Cockhill, Jhamandas and Boegman (1991)[6] Beninger, Kühnemann, Ingles, Jhamandas and Boegman (in preparation)[3]
Bilateral ibotenic acid NMB lesions	↓	↓	Beninger, Kühnemann, Ingles, Jhamandas and Boegman (in preparation)[3]
Unilateral phthalic acid NBM lesions	↓	–	Mallet, Beninger, Jhamandas and Boegman (1992)[12]
Bilateral muscimol infusions into the NBM			
0.01 μg in 0.5 μl	–	–	Beninger, Ingles, Mackenzie, Jhamandas and Boegman (1992)[2]
0.1 μg in 0.5 μl	↓	–	
1.0 μg in 0.5 μl	↓	↓	
Age (8 months vs. 16 months old)	↓	–	Biggan, Ingles, and Beninger (in preparation)[5]
Systemic scopolamine (8 months old)			
0.1 mg/kg	–	–	Biggan, Ingles, and Beninger (in preparation)[5]
0.4 mg/kg	↓	–	
0.8 mg/kg	↓	↓	
Bilateral scopolamine amygdala infusions			
8 μg in 0.5 μl	–	–	Ingles, Beninger, Jhamandas and Boegman (in press)[11]
24 μg in 0.5 μl	↓	–	
72 μg in 0.5 μl	↓	↓	

6. DISCUSSION

A multitude of paradigms has been used to assess reference and working memory in animals including the radial maze[1,18], the T-maze[4,7], and delayed matching-to-sample[16,17] to name but a few. The double Y-maze has an advantage over other paradigms in that the reference and working memory components are identical in all respects, except for the type of memory required for successful performance. Hence, if working but not reference memory performance is impaired following some experimental manipulation, one can be fairly certain that the impairment is due to decreased mnemonic function, rather than to impairments of perceptual, motor, or motivational systems. For example, if performance deficits are due to an impairment in perception rather than a deficit in memory, one would expect an impairment on *both* reference and working memory components.

Another advantage of the double Y-maze is that the number of reference and working memory errors can be compared directly since the probability of making each type of error is the same. Although the reference and working memory components of the radial maze task[1,18] are similar in terms of demands on motivation, perception and motor performance, a problem exists in comparing relative impairments since the probability of making each type of error is not equal. In one version of the radial maze task, entries into arms which never contained food constitute reference memory errors whereas re-entries into already-visited arms constitute working memory errors[8]; thus, in an 8-arm maze the probability of making a refer-

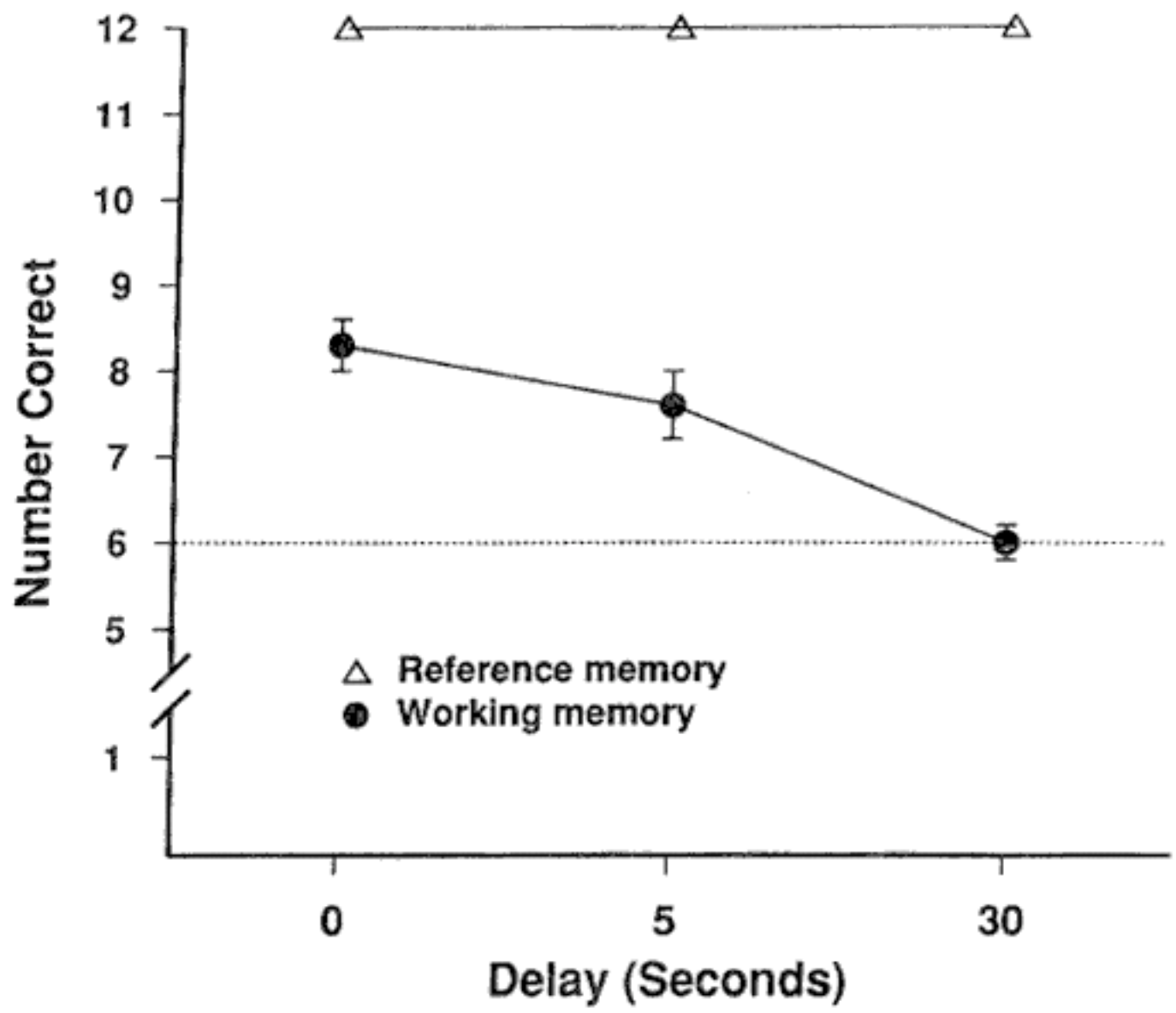

Fig. 3. Mean (± S.E.M.) number of correct working and reference memory choices on the double Y-maze as a function of delay. Data represent means of eight rats which were tested for 15 days, 12 trials per day, with 4 trials at each delay. A delay-dependent decrease in working memory but not reference memory performance was observed. The dashed line at 6 choices represents chance performance. (Data are from Biggan et al.[6])

ence memory error on the first choice of a session is 0.5 (if only half of the arms are baited). Conversely, the probability of making a working memory error on the first choice is 0 because there are no arms to re-enter. On the second choice the probability of making a reference error remains 0.5, and the probability of making a working memory error becomes 0.25 *if* the first choice was correct. However, if the first choice resulted in an error, then the probability of making a working memory error on the second choice remains 0. On the third choice, interpretation becomes even more complex. The probability of making a reference memory error is still 0.5, but the probability of making a working memory error becomes: 0.5 if the first and second choices were both correct, 0.25 if either of the first or second choices were correct, and 0 if neither were correct. A comparison between reference and working memory becomes even more difficult when one considers whether or not a re-entry into a never-baited arm should be scored as a reference or a working memory error. Although the radial maze has the ability to partial out the effects of an experimental manipulation on reference and working memory, a direct comparison between the two is not easily accomplished.

It does not appear that non-mnemonic variables such as odour cues, general algorithms, or response chains[13] play a major role in directing performance in the double Y-maze. Odour cues cannot be utilized in the reference memory component since two arms are always traversed on this part of any given trial. Hence, there are always two arms which will contain the animal's scent from the previous trials. Second, intra-maze odours cannot be used in the working memory component since the choice is made in the second Y-maze and accurate performance depends entirely on the start position of the first Y-maze. In addition, since small pieces of food are scattered beneath the floor of the maze, the animal cannot simply choose the arm which smells of food. Response chains are prevented by the random placement of the rat in the start box for the reference memory component. That is, rats are not allowed to determine their own pattern of food collection. Moreover, it does not appear that any response strategies which do not require the use of memory affect performance in the working memory component since it has been shown that the working memory component of the double Y-maze is susceptible to delay dependent interference[6]. A delay interval likely would not have decreased accuracy if a non-mnemonic response strategy had been adopted.

It is interesting to note that at least one strategy does appear to play a minor role in directing choice performance in the double Y-maze. As mentioned earlier, rats tend to learn the working memory component in fewer trials if they are rewarded for the sequences A-C-A′ and B-C-B′, as opposed to being rewarded for the sequences A-C-B′ and B-C-A′ (See Fig. 2). It has been shown that rats spontaneously (i.e., without training) alternate when searching for food and this probably explains the observed phenomenon[13]. This does not pose a problem in the assessment of memory since successful spontaneous alternation still requires the use of working memory.

Problems with the double Y-maze may exist when: (1) both reference and working memory components of the double Y-maze are impaired, (2) acquisition rates for each component are not equal, and (3) latency to make a choice is increased as a function of the experimental manipulation. First, when both reference and working memory components are impaired, there may exist a deficit in motor, perceptual or motivational function in addition to, or instead of, a mnemonic deficit. Although it is possible that reference and working memory are both impaired, it is not possible to rule out other explanations without conducting additional tests using paradigms sensitive to changes in non-mnemonic functions. On the other

hand, when only a working memory deficit is seen, changes in non-mnemonic variables seem unlikely.

Second, whereas the reference memory component is easily acquired in only a few sessions, a relatively large number of sessions are required before criterion is reached on the working memory component. Although this remains an open question, it is possible that the differential effects on reference and working memory of a given experimental manipulation are merely due to a difference in task difficulty. That is, difficult tasks may be more sensitive to interference.

Last, experimental manipulations such as excitotoxic lesions which have the potential to induce motor hypo-activity may increase the latency to travel from the choice point of the first Y to the choice point of the second. Consequently, observed deterioration of the working memory component may not actually be caused by a deficit in mnemonic function, but may be related to an increase in retention interval. Although this problem has not yet been resolved, plans are currently underway to measure travel time in the maze.

In conclusion, the double Y-maze is a promising new tool for assessing memory in rats. Future research should attempt to further refine the task by systematically manipulating various parameters such as number of trials per session, delays, and massed versus spaced trials in order to further identify the influence of these variables on acquisition and performance.

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QUICK PROCEDURE

Pre-training

A. Handle the rats and deprive them of food.

B. Introduce the rats to the food reward in their home cages.

C. Habituate the rats to the maze for 3 days.

Training/Testing

D. Bait the maze with food reward.

E. Position the doors.

F. Randomly place the rat in one of the start boxes and remove the door.

G. Remove the central door once the animal reaches the centre of the maze and the food is consumed and place another door behind the rat.

H. Remove the rat when the second goal box is reached and the food is consumed.

I. Repeat steps D to H for the desired number of trials which constitutes a session. Continue until criterion is reached or test data are collected.