Review

An overview of the tasks used to test working memory in rodents

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Abstract

In rodents, working memory is a representation of an object, stimulus, or spatial location that is typically used within a testing session, but not between sessions, to guide behaviour. In this review we consider a number of the tasks used to assess this type of memory in the rodent, and highlight some of their limitations. Although the concept of working memory as applied to rodents has its origin in the experiments of David Olton and Werner Honig in the 1970s, many earlier experiments assessed the same type of memory under the guise of delayed reaction or alternation paradigms. We revisit these early tasks, and also consider the nature of working memory used on maze tasks, operant box based tasks, and non-spatial delayed non-matching to sample paradigms.
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Keywords: Working memory; Delayed alternation; Delayed non-matching to sample; Animal cognition

Contents

What is it that we rat runners [psychologists who work with rats] still have to contribute to the understanding of the deeds and misdeeds, the absurdities and the tragedies of our friend, and our enemy-homo sapiens? The answer is that, whereas man's successes, persistences, and socially unacceptable divagations—that is, his intelligences, his motivations, and his instabilities—are all ultimately shaped and materialised by specific cultures, it is still true that most of the formal underlying laws of intelligence, motivation, and instability can still be studied in rats as well as, and more easily than, in men [1].

E.C. Tolman (1945)

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The purpose of this review is to consider the different tasks used to assess working memory in the rodent, and to highlight some of their limitations. As the quote above suggests, there are clear advantages to studying basic psychological processes in the rodent. However, there can also be difficulties, and these are particularly evident in the study of memory. Do rats have episodic memory? Can one truly differentiate working memory from other types of short term memory in rodents? Or is 'working memory' an artificial distinction having to with how a short term memory is used? These remain, for the moment, open questions.

The approach taken in this review will be to start by considering exactly what is meant by working memory in the rodent. Next, we will focus on the tasks themselves. These tasks are commonly used neurobiology and psycho-pharmacology studies of memory, although a full review of these neurobiological studies is beyond the scope of the current review. We will conclude with a few words on the usefulness of different tasks for the assessment of working memory.

1. What is working memory in a rodent?

The term 'working memory', as applied to animal cognition, originates in the experiments David Olton and Werner Honig in the 1970s. Olton and Samuelson [2] devised a classic task for assessing memory in the rodent, the radial arm maze (Fig. 1A). The maze is comprised of eight arms radiating from a central platform. In this maze, the rat is placed on the centre platform, and a food reward is available at the ends of each arm. Olton and Samuelson observed that rats would retrieve the food from each arm, and quickly learned to visit all the arms without re-entering a previously visited arm. In their first eight choices on the maze, trained rats typically entered >7 correct arms before making an error. In a series of experiments, the authors ruled out the possibility that the rats visited the arms in a particular order, used odour marking of visited arms, or alternative intramaze cues. This suggests that the rats were able, in a single session, to remember which arms they had visited. This, for Olton et al. [3], was *working memory*:
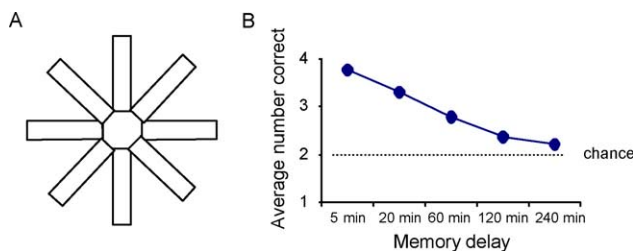

Fig. 1. (A) Schematic of the eight-arm radial maze. (B) Performance decrease associated with the introduction of different delays between the fourth arm choice, and the remaining arm choices. Data based on Bolhuis et al. (1996).

memory that allows the animal to remember which arms it had visited in a session. On the next day, this memory is no longer relevant, as all of the maze arms are baited again.

Honig [4] argued that working memory is a representation of a cue over a delay period in which the cue is not present, to make a subsequent response. This memory can be distinguished from reference memory (a long-term association between stimuli or a stimulus and a response) by its transience. A working memory functions on a particular trial, but then must be forgotten or ignored on subsequent trials.

For humans, the concept of working memory is more explicit. Baddely and colleagues [5,6] have proposed a model of working memory comprising a central executive, and two sub-systems, a visual–spatial sketch pad, and a phonological loop. Clearly, the latter is not an obvious feature of rodent cognition, and so this model is difficult to apply across species. A similar challenge in applying concepts derived from human memory findings to non-humans is found in recent attempts to develop episodic-like memory tasks for rodents (see [7,8]).

A recent definition by Eichenbaum and Cohen [9] has emphasised the 'working' aspect of this type of memory. These authors define working memory as a type of short-term memory that involves active manipulation by the individual. Thus, examples of working memories might include the holding on to an item that would be compared to recently presented items, or the storing of digits as one counts backwards from 100 by 5's. Importantly, working memory may not necessarily be associated with long term memory, and there is lesion evidence suggesting that the two systems may depend on different brain systems.

In this review, we will define working memory as *a short term memory for an object, stimulus, or location that is used within a testing session, but not typically between sessions*. It is distinguishable from reference memory, which is a memory that would typically be acquired with repeated training, and would persist from days to months. Reference memory is often a memory for the 'rules' of a given task, for example, that a bar press produces a food pellet, or that a water maze contains a hidden platform. Working memory, in contrast, is typically a delay-dependent representation of stimuli that are used to guide behaviour within a task. (Whether this delay-dependence is an active or passive process is an open question.) For Olton et al. [3] working memory is distinct from reference memory because it uses flexible stimulus–response associations, is sensitive to interference, and represents temporal order.

It should be noted, however, that the distinction between working and reference memory may not be absolute. Morris et al. [10] have suggested that animals may be able to discriminate between information gained on a recent trial as opposed to information gained on other trials, and use the former to guide behaviour. This view does not assume that working memory is qualitatively different from longer term memories.

Likewise, it may be difficult to distinguish between working memory and other forms of short term memory in the rodent. *It is possible that this distinction simply refers to how the memory is used, rather than its nature*. Working memory is a short term memory that, once used, should be forgotten or ignored. Presumably, it is useful for the rat to forget which arms of the maze it visited yesterday, or for us to forget where we parked our car last week. The duration of these working memory may depend on how long they will be useful and the salience of the to-be-remembered stimuli. Other short term memories may not require forgetting. These memories may be important or may not interfere too much with subsequent encoding, and thus may provide the basis for longer term memories. Working memory may not actually be a type of memory, but a type of forgetting.

In what follows we consider different tasks used for assessing working memory in the rodent. We briefly review early studies on delay memory, and then examine maze tasks, tasks run in operant chambers, and non-spatial tasks.

2. Early studies on delay memory in rats

Although Olton and Honig were the first researchers to apply the term *working memory* to the animal's short-term storage of information, earlier experimenters had devised tasks for assessing this type of memory in animals. These studies focussed on developing tasks to see how long a rat could remember a stimulus that was not present. These were often referred to as 'delayed reaction' paradigms, and a useful review of this early literature may be found in [11].

Walter S. Hunter provided one of the first tests of short-term memory in the rodent [12]. He tested rats, racoons, dogs, and children in a task where 'the determining stimulus is absent at the moment of response' (p. 1). The rat apparatus consisted of a chamber in which a light appeared behind one of three screen doors. The rats began each trial in a release box that faced the three doors. A light was briefly illuminated behind one of the doors and then extinguished. The rat's task was to run to the door in which the light had appeared. If it did so correctly, it received a small piece of bread and milk.

Hunter observed that rats were able to remember which door had been illuminated over a delay of up to 10 s. However, during these delays, the rats in almost every instance oriented towards the correct door. Thus, the rats 'bridged' the delay by orienting towards the correct stimulus immediately after it was presented. This strategy does not allow one to conclude that the rat is using memory—an internal representation of the previously presented stimulus—to guide its behaviour.

A further attempt to assess memory over short delays was made by McAllister [13]. He trained rats on a conditional alternation task in which the rat learned to go down either the left or right alleyway in rectangular shaped maze depending on what stimulus was presented in the start area of the maze.

Different rats were trained with different types of stimuli, and these included a light, an auditory stimulus, and a tactile/kinaesthetic stimulus. Delays between the stimulus presentation and the response were increased by increasing the distance that the rats had to run beyond the start area before they could choose one of the alleyways. McAllister found that some evidence for the use of a overt bodily orientation towards the correct alleyway in initial training trials, but this disappeared with additional testing. Furthermore, rats still responded correctly when the paths that the rats took were disrupted by adding an S-shaped box after the start area. For McAllister, these findings suggested that rats must have solved the task by relying on an 'intraorganic cue'.

Another example of the type of task used to assess delayed response was the maze developed by McCord [14]. He placed rats inside a square black chamber that had doorways on each wall. Each doorway had a different visual pattern on a white background, and beyond the doorway was a platform upon which the rat could find a food reward. From outside the apparatus, the experimenter would place his hand through one of the four doors, and wave a small food dish at the rat. The rat was contained in a small birdcage like apparatus within the chamber. The experimenter's hand was withdrawn, a delay would ensue, and then cage would be lifted, allowing the rat to jump through the door of its choice. Only by jumping through the door in which the experimenter had waved his hand would the rat obtain reinforcement. McCord observed that rats could remember the correct door with delays up to 6 min, and they did so without any postural mediation of the delay.

These early studies assess the type of memory that today would be referred to as working memory. As is evident, even in these early studies researchers were aware that the development of valid memory tasks required the exclusion of postural mediation of the to-be-made response. Other early studies assessed memory over short delays using alternation paradigms. As these may be considered spatial working memory tasks, we will consider them in Section 3.

3. Maze tasks for assessing spatial working memory

Since the first maze study by Small [15], many experimenters have taken advantage of the rats' penchant for narrow, winding passageways when developing tasks. Many early studies sought to define the precise sensory cues that rats used to solve mazes. Other tasks required rats to remember a location or set of locations, and either approach or avoid these locations subsequently. In this section, we consider tasks that are used to assess this 'spatial' working memory.

3.1. Delayed alternation

Delayed alternation problems capitalise on the rats' tendency to choose alternative maze arms or locations when
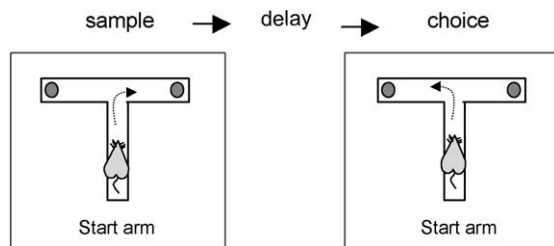
Fig. 2. Delayed alternation on a T-maze. On the first or sample run, the rat is placed on the stem of the T-maze and permitted to enter one of the arms. The rat may then be removed from the maze for a delay period. After the delay, the rat is returned to the stem of the maze, and, will typically choose the alternate arm of the T.

rats are re-exposed to an apparatus. As the animals must remember their initial response to select this alternative response, this is a short-term memory task. According to Loucks [16], the delayed alternation problem can trace its origins to a study by Carr [17].

The T-maze. Perhaps the most common version of the delayed alternation problem is the T-maze (Fig. 2). Tolman [18] was one of the first experimenters to use a T-maze, and he described the 'very pronounced' tendency for rats to alternate arm choices on repeated trials. The task works as follows: a rat is first placed at the base of the T, and it runs up the stem, and enters one of the arms of the T. Here the rat may obtain a reward at the arm's end. The rat is picked up by the experimenter, and replaced at the base of the T. Typically, the rat will run up the stem and enter the arm of the T it had not entered on its first run. This is alternation. Rats (and other animals, for review, see [19]) will alternate without reinforcement, and this is referred to as spontaneous alternation. Rats may also obtain reinforcement at the maze arm ends, and if entry to the alternate arm is prevented on the first run, the task is referred to as a 'forced-choice T-maze alternation'. Interposing a delay between the first and second run makes this a delayed alternation task.

The tendency to alternate is a curious one, and is worthy of comment. According to Thorndike's Law of Effect, an animal that is reinforced for a given behaviour should be more, not less, likely to repeat that behaviour. Thus, rats should tend to re-enter the arm of the T in which they find reinforcement, and not to choose the alternate arm. To account for this, Hull [20] proposed the concept of reactive inhibition. Essentially, when a rat turns one way on the T maze, a certain amount of reactive inhibition is generated which makes it less likely for that same turn to be repeated immediately. Montgomery [21], however, provided data that argue against this view. Using a plus-shaped maze, she found that rats tended to alternate spatial locations, and not body turns. By her account, alternation is a form of exploratory behaviour by the rat. Related views hold that alternation is due to stimulus satiation [22] or attention to stimulus change ([23]; for reviews, see [19,24]).

Although it is perhaps not fully understood *why* rats alternate on the T-maze, the task has been used in a variety
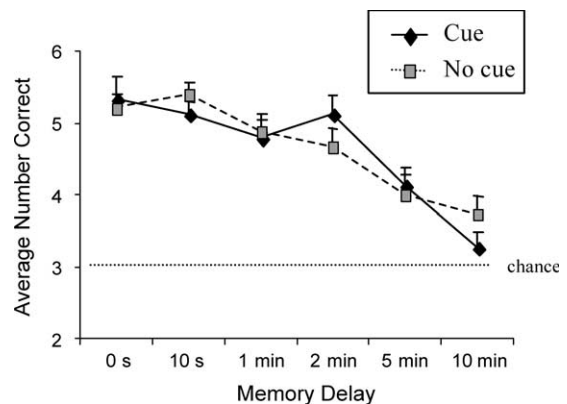

Fig. 3. Delay-dependent performance on the T-maze. Rats were tested in the presence (Cue) and absence (No Cue) of extra-maze landmarks with delays of 0–10 min between sample and choice runs on the T-maze. By Dudchenko PA. How do animals actually solve the T-maze? Behav Neurosci 2001;115:850–60. Copyright 2001 by the American Psychological Association.

of learning and memory studies, and alternation performance is a particularly sensitive to the effects of hippocampal disruption. Hippocampectomised rats perform at chance level with delays as short as 15 s. (e.g. [25]), and this impairment has been evident in even the earliest studies with this apparatus ([26]; for review, see [3]).

In a recent study we tested the duration of memory on the T-maze ([27]). Ten rats were tested on a T-maze with delays up to 10 min. A delay-dependent decrease in memory was observed, with above chance performance at 5 min, but performance at a chance level with 10 min (Fig. 3). Memory over longer delays was observed in an earlier study by Petrinovich and Bolles [28]. Using a water-reward, they sought to test the limits of delayed alternation memory on the T-maze, and found that the best 7 of their 16 rats were able to alternate with a 30 min delay between runs. A subset of these animals alternated at an above chance level with longer delays, and 1 rats was above chance with a 5.5 h interval between runs.

Identifying the nature of the rats' memory on the T-maze has proven to be a more complicated problem. In their review of spontaneous alternation on the T-maze, for example, Richman et al. [19] suggest:

The appeal of SAB [spontaneous alternation behavior] lies in its reliability and apparent simplicity. The reliability is real, but the simplicity is deceptive (p. 358).

When the rat alternates, it may do so based on memory for a number of different types of information. Typically, it is assumed that rats solve the T-maze by using remembering the location of the most recently visited arm based on its spatial relationship with extramaze landmarks. This is an allocentric (world-based) spatial memory. The rat, however, may alternate based on a directional sense, first going west, for example, and then going east. Alternatively, the rat might use a response strategy—remembering which turn it

has made (e.g. left), and make the opposite turn on the subsequent trial. Finally, if subtle odour cues are left on the maze arms, the rat may detect the arm that it has most recently entered by its smell.

A study by Douglas [29] attempted to determine the cues used in alternation performance. After a variety of manipulations the author concluded that rats 'have a sense of direction or position in space' (p. 171) that can be used to remember which arm of the T has been most recently sampled. Recent data [30] has supported this conclusion.

Other data have shown that rats trained in the absence of visual landmarks performed as well as those with landmarks, even across delays [27]. However, rats tested with landmarks did use these, as rearrangement of the landmarks during a delay between the first and second runs produced an impairment in performance. These results suggest that the could use a variety of strategies to remember which arm they have entered most recently. This is significant because it suggests that interpretation of deficits (or lack thereof) following lesion of pharmacological manipulations cannot be exclusively ascribed to memory for extramaze spatial relationships.

Delayed alternation on other mazes. Delayed alternation has been assessed on mazes other than the T-maze. A study by Dennis [31], for example, used square track with a start box on one side and a goal box on the other (see Fig. 4A). Rats would leave the start box and traverse one side of the square track to reach the goal box. On a subsequent trial, they tended to choose the alternate side of the track to reach the goal box 82% of the time. If a second square track was added to the first track, such that the rat would have to traverse both to reach the goal box, there was no tendency for alternation from one track to the other. This suggests that the alternation is not based on a tendency to turn in the direction opposite the last response. Rather, it indicates that rats may alternate on the basis of a context-specific memory.

Another alternation task is that of Ladieu [32]. The goal of this study was to test the memory of albino rats for locations on the apparatus shown in Fig. 4B. Rats started in the centre of the box, and were permitted to enter one of the side compartments of the apparatus, where they found a food reward. The rat were replaced in the center compartment, and this was closed off by a door. The rats remained in the center compartment for delays up to 480 min. After the delay, the door was opened and the rat was permitted to select either side compartment. Only the side compartment that the rat had not entered on its first run contained reward. Impressively, rats alternated at an above chance level with delay of 120 min, but not at 480 min.

A final example of an alternation apparatus is the maze developed by Whishaw et al. [33]. They sought to develop a task in which the rat would not have to be handled during the first and second runs on a maze. The authors constructed mazes with two arms that were connected by either a cylindrical environment, or a small square environment. The two maze arms were oriented at 180° to one another, and the rat was placed in either the connecting cylinder or square, and allowed two arm choices. Regardless of the number of food pellets found at the end of the maze, rats tended to alternate on both apparati.

Although alternation is a robust phenomenon, rats tend not to alternate at an above chance level when the configuration of a T-maze is changed such that two arms of the T are parallel to one another [34]. This suggests that it is the spatial distinctiveness of the two goal arms that contributes to the rats' tendency to alternate. However, spatial distinctiveness alone is insufficient to produce alternation. Whishaw and Pasztor [35] observed that rats would alternate 80% of the time when walking between two food locations, but would choose the same location on 80% of the trials in which the rat had to swim to a platform on which food was available. This is striking, as it implies that commonly used tasks such as Morris water maze and the radial arm maze may tap quite distinct behavioural propensities in the rodent.

3.2. The radial arm maze

We have described the basic radial arm maze above, but will now consider some of its variants. A complete review of radial arm maze studies can be found in Foreman and Ermakova [36].

Delays on the radial maze. In their initial experiments with the task, Olton and Samuelson [2] tested rats on a version of the maze where a delay was introduced after the rats had made their third arm choice. After a 1 min 'confinement' to the center platform by guillotine doors, the rats were permitted to complete their maze arm choices. Introducing this delay after the third arm choice had no effect on the accuracy of the subsequent choices, and the rats still made an average of 7.7 correct responses in the first eight choices. Increasing the delay to 'at least 2 min' (p. 110) likewise did not produce an impairment in performance as the rats made an average of 7.6 correct
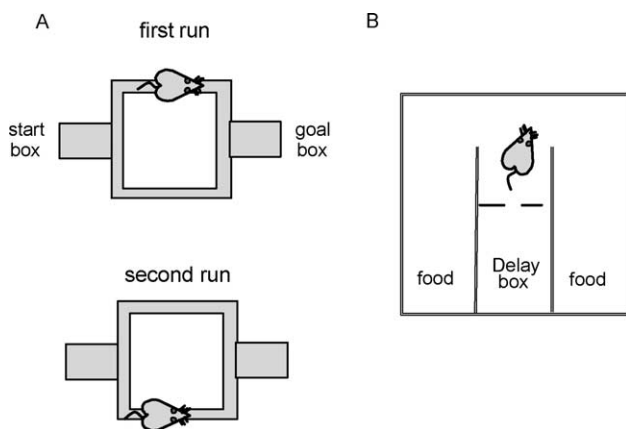

Fig. 4. Early alternation tasks. (A) Dennis [31] observed that rats would alternate paths from a start box to a goal box on square track. (B) Maze apparatus used by Ladieu [32] to test the duration of rodent memory.

responses in their first 8 choices. The authors suggest that the memory for previous arm choices 'is obviously much longer than the few minutes tested here' (p. 110).

Using a similar procedure Suzuki et al. [37] introduced a 2.5 min delay between the third arm choice and the subsequent choices. After the delay, the rats readily visit the maze arms they had not previously entered, and made an average of 4.3 correct choices from the five remaining arms. Importantly, Suzuki et al. also show that rats rely on the spatial relationships between extramaze landmarks when solving the task, as rearrangement of these landmarks during the delay period resulted in chance performance following the delay.

How long can rats remember which arms they have entered? Bolhuis et al. ([38]; see also [39]) studied the duration of memory for visited arms on the radial arm maze and found that rats were significantly impaired with a 60 min delay between the fourth and subsequent arm choices, and performed at a chance level with a 120 min delay (see Fig. 1B).

Working and reference memory. Jarrard et al. [40] developed a version of the radial maze to test working and reference memory at the same time. In their version of the task, only four maze arms are baited. The same maze arms are baited each day, and across sessions, the rats learn to ignore the remaining four arms, which never contain reward. This is the reference memory component of the task, and entry into a never-baited arm is considered a reference memory error. Within a training session, re-entry into one of the four baited arms would be considered a working memory error. Rats with hippocampus lesions exhibit working memory errors (and some reference memory errors) on this version of the task, but can learn an intramaze, 'cue' version of the task to the same level as control subjects.

Jarrard [41] have also tested match-to-sample version of the radial arm maze. In this task, the rat is permitted to run down one arm of the maze to obtain a reward. It then returns to the central platform, and is confined there for a delay period. After the delay, the doors in front of all the arms are lowered, and the rat's task is to return to the arm it initially obtained food on. On different days, different arms serve as the to-be-remembered stimulus. Hippocampectomised rats exhibit a delay-dependent impairment on this task.

Other variants. The number of arms can be varied on the maze. A four-arm maze is often referred to as a plus maze, and an early study by Olton and Feustle [42] suggested that rats with fimbria–fornix lesions were impaired on a non-spatial version of this task. Other versions include mazes with 12 arms (e.g. [43,44]), 17 arms [45] and 24 arms (e.g. [46]). Accuracy on these tasks can be impressive. In the 17 arm maze, for example, rats make ≥ 14 correct responses in the first 17 choices [45].

Summary. On the traditional eight arm maze and its variants, rats reveal their working memory for the arms that they have visited by not re-entering them. Typically, they do

so by relying on their memory for the spatial location of visited arms relative to the extramaze landmarks in the testing environment. The radial arm maze may be related to the alternation task described in Section 2; Olton and Samuelson [2] have suggested that excellent performance of rats on their radial maze 'is presumably a reflection of spontaneous alternation' (p. 113).

3.3. Working memory in the Morris water maze

The Morris water maze task requires rats to find a submerged platform in a large, circular pool of water [47]. In the task as it is typically run, the submerged platform remains in the same location in the pool across days, and rats solve the task by learning the spatial relationships between the platform location and the extramaze landmarks in the testing environment [48]. This version of the task is a reference memory task (for reviews, see [49,50]).

Working memory versions of the task have also been developed. Morris et al. [10] tested rats on the water maze with the platform in a *different* location on each day. They observed that the rats learned the location of the hidden platform in one trial, as evidenced by a shorter latency to swim to the platform on a second trial. Hippocampecto-mised rats did not show this savings. In a more recent version of this task, Steele and Morris [51] gave rats four trials per day in the water maze, with a different location each day. They termed this a delayed matching to position (DMP) task, and varied the delay between the first (sample) trial of the day, and the second trial. The savings in latency to swim to the hidden platform between the first and second trials on a given day was used as a measure of the rats' memory for the platform location. Rats with lesions of the hippocampus and dentate gyrus were impaired at all delays (15 s–2 h) on this task, whereas intra-hippocampal blockade of NMDA receptors only affected memory delays of 20 min and 2 h.

Another working memory version of the water maze has been developed by Buresova et al. [52]. They constructed a radial arm maze within a water maze, and the rat's task was to swim to the end of each arm to a submerged bench. The bench provided refuge from the water for 20 s, and then it collapsed, forcing the rat to swim to a different maze arm. Bolhuis et al. [53] found that trained rats made 7.8 correct arm choices in their first eight choices. In this study memory for the visited arms was tested by introducing a delay between the fourth and fifth arm choices. Delays ranged from 40 to 1280 min, and performance decreased with increasing delays, reaching a chance level at a delay of 640 min.

4. Delayed non-matching to sample with objects, odours

Delayed non-matching to sample (DNMS) tasks require a rat to remember a stimulus over a delay in which that stimulus is no longer present. Following the delay, the rat is

presented with the to-be-remembered stimulus and an alternative, and the rat is reinforced for making a response towards the alternative stimulus. In delayed *matching* to sample (DMS) tasks rats are rewarded for selecting the to-be-remembered stimulus. The origins of DN/MS tasks lie in the delayed reaction tasks of Hunter [12] described above.

Spontaneous exploration. A variant of the DNMS task for rodents capitalises on the tendency of rats to explore novel stimuli and environments. In one version of this task, Aggleton [54] trained rats on a Y-shaped maze where boxes that contained different stimuli could be fitted to the end of each maze arm. On a given trial, two of the boxes would be identical, and one box would be novel. The rat would start in one of the identical boxes, and after a delay, it would be reinforced for running to the box that differed from its start box. Thus, this was a non-matching to sample task. Aggleton introduced delays by substituting the 'sample' box with a featureless box after 20 s, and rats exhibited delay dependent performance on this task.

A task developed by Ennaceur and Delacour [55] looked at the spontaneous exploration of objects. In the task in its simplest form, a rat is presented with an object (A) or pair of identical objects in a small arena (Fig. 5). After a brief exposure period, the rat is removed from the arena and a delay ensues. The rat is then brought back to the arena, in which a duplicate of object A and a novel object (B) are to be found. The rats' natural tendency is to explore the novel object B more than the (presumably) familiar object A. Ennaceur and Delacour tested delays of 1 min–24 h between the initial exposure to object A, and the subsequent exposure to both A and B. Rats showed a preference for the novel object with delays of up to 4 h, and this preference decreased after 24 h. When the initial exploration of object A was limited to 20 s, a preference for the novel object was not significant after a 1 h delay. In a subsequent experiment using a two identical objects during the sample phase of the task, rats spent significantly more time exploring the novel object with a 60 min delay, but not with a 24 h delay [56].

A careful examination of this task was done by Dix and Aggleton [57]. They observed that, in the test phase of

the standard object exploration task, rats tended to explore the novel object significantly more only in the first 2 min of the session; thereafter they appeared to habituate to the object. The authors also found that the spontaneous exploration paradigm could be used to test memory objects in specific contexts and specific locations. Thus, the same apparatus could be used for assessing both spatial and non-spatial memory.

Delayed non-matching to sample with objects. Rothblat and Hayes [58] describe a version of the DNMS task with trial unique stimuli. (The use of trial unique stimuli is distinguished from the repeated use of a smaller number of familiar stimuli, as the former places demand on recognition memory, while the latter may rely more on recency memory [59]). The task worked in the following way. Rats were presented with a sample object at the end of a straight runway. The rat would displace this object to obtain a food pellet. A 10 s delay ensued, after which the rat again ran down the runway and encountered both the sample object and a novel object. Reward was provided if the rat selected the novel object. The authors found that overall, the rats chose the novel object on between 70 and 80% of the trials, and this percentage decreased with 30 and 120 s delays.

A task by Mumby et al. [60] similarly used trial unique stimuli, and observed rapid learning of the DNMS task. Their apparatus consisted of a straight runway with a two goal areas on each end. Rats were restricted to the center portion of the runway by two guillotine doors, and the task began with one of these doors opening to provide access to an object at the end of the runway. In this sample phase of the task, the rats displaced the object to find a food pellet below it. Following a delay, the other door was opened, and the rat could find the sample object and a novel object at the opposite end of the runway. If the rat displaced the novel object, it received a food pellet.

Rats were initially trained with a 4 s delay between the sample and choice phases of the task. Mumby et al. then tested their rats with delays up to 600 s and observed a delay-dependent decrease in choice accuracy. On control tests with duplicate objects for the sample and choice phases of the trial, the rats performed as well as when the same object was re-used, and thus it is unlikely that any odour 'marking' strategy was employed in the rats' recognition of the sampled objects.

An object DNMS was also used by Kesner et al. [61] to examine the contribution of hippocampus to working memory, and an excellent review on the use of these tasks to role of the hippocampus in object recognition memory can be found in [59].

One issue in the object DNMS task is the possibility that odour cues may be left on the objects by the experimenter. In the choice phase of the task, if the experimenter systematically handles the sample object last when placing it in the testing arena, it is possible that rat may use this cue (instead of the memory of the sample object) to solve the task [62]. An explicit assessment of this possibility was
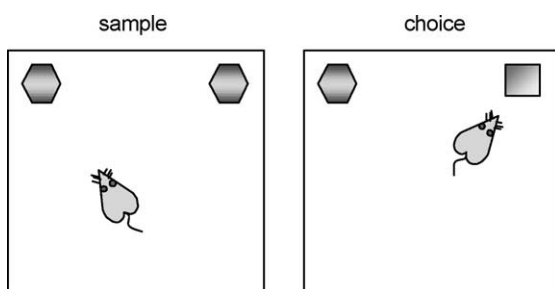

Fig. 5. The spontaneous exploration task of Ennaceur and Delacour [55]. A rat is presented with an object or pair of identical objects (left plot) which it is allowed to explore. Following a delay, the rat is presented with the previously presented object and a novel object (right plot), and the rat tends to demonstrate its memory for the previously presented object by spending more time exploring the novel object.

done by Mumby et al. [63]. They tested rats with the same cue on the sample and choice phases of their DNMS task. The cues in the choice phase only differed in the recency with which they were handled. Rats *were* able to learn the solve the task on the basis of the most recently handled object, but were only able to do this with a 4 s delay. Extension of the delay to 15 s resulted in chance performance. In a second experiment, the authors showed that placing the sample object into the arena after the novel object (for the choice phase of the trial) did not confer any advantage in learning from pseudorandom handling of the objects. Thus, rats can detect odour cues left by the experimenter, but this is a transient cue that does not appear to be very salient.

Delayed non-matching to sample with odours. Otto and Eichenbaum [64] developed a continuous DNMS task using odours. Rats were trained and tested in a square chamber that contained an odour port and a water port. When the rats made a nose poke of at least 500 ms duration to the odour port, one of 16 odours was presented. If the odour differed from the last odour that had been presented, a nose-poke to the water port, located just above the odour port, produced reward. If the odour was a repeat of the last odour that had been presented, nose-pokes to the water port were not reinforced.

Recent variants of this task have used a 'digging' response to test working memory. Wood et al. [65], for example, trained rats to dig in cups of sand scented with household spices. In this task, rats were presented with individual cups of sand on an open platform. On cup was presented at a time, and a food reward was only available in cups of sand whose scent differed from the immediately preceding cup. Rats readily learned to attend to the scent of the sand and dig in the non-matching cups, but not cups with scents that were repeated.

Another variant of this task tested the rats capacity to remember a list of previously presented stimuli. Few studies have assessed the rats memory capacity, with the exception of Steele and Rawlins [66,67], who tested memory for distinctive goal boxes on a Y-maze. Dudchenko et al. [25] trained rats on a discrete trial, odour non-matching to sample task (see Fig. 6). Rats were first presented with a cup

of sand scented with a household spice. A food reward was obtained by digging in this cup. After a short delay, the rat was again presented with a cup of sand scented with the first odour, and a cup of sand scented with a novel odour. Reward was only available in the cup of sand with the novel odour. After another delay, the rat was presented with three cups of sand, two of which had been previously presented, and one which contained a novel odour. As before, reward was only available in the cup contained the novel odour. In this way, the rats' memory for multiple stimuli could be assessed. Rats readily performed this task, and accurate performance has been observed even when memory for 24 odours was required [25,68].

Odour digging tasks have also been used to test the rats' memory for the *sequence* in which odours are presented [69–71]. The results of these studies are consistent in showing that memory for odour sequence, as opposed to simple recognition, requires an intact hippocampus.

Do spontaneous exploration and DNMS measure the same thing? Although rats may remember the same stimulus (for example, an object) in a spontaneous and a reinforced DNMS task, evidence from primate studies suggest that these tasks are not equivalent. In particular, Nemanic et al. [72] found the partial hippocampal lesions in rhesus monkeys produce a significant impairment in a spontaneous (visual) exploration task with delays of 60 s. These same monkeys were unimpaired on a object DNMS task with delays of up to 600 s. These and other results [73] suggest that the memory for actively encoded stimuli in a DNMS task requires different brain regions than memory for passively encoded stimuli in a spontaneous exploration task. Thus, how a stimulus is encoded may determine how it is represented in the brain.

5. Operant tasks for assessing working memory

Other versions of the DNMS task have been developed for the operant chamber. In an early version of these tasks, Pontecorvo [74] developed a continuous non-matching task using a dim and bright lights as the discriminada. Each stimulus was presented for 5 s, and bar presses were
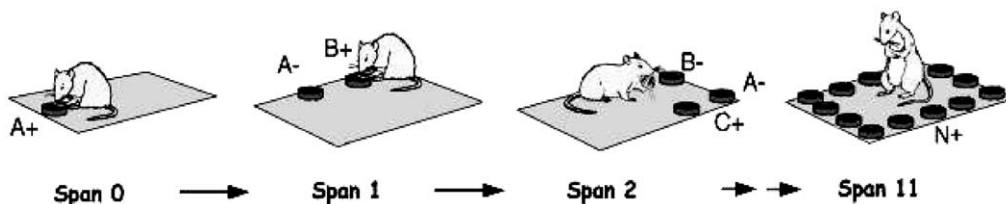

Fig. 6. Odour 'span' task. A rat is first presented with a cup of sand that is scented with a specific odour (A). After a delay, the rat is presented with a cup of sand scented with that same odour, and one scented with a different odour (B). Food reward is available only in the cup containing the non-matching odour (B+). Additional cups of sand, scented with different odours, are presented to the rat and its task is to remember which scents it has previously sampled. The rat's span is the number of odours it can correctly remember before making an error. Figure by Dudchenko PA, Wood ER, Eichenbaum H. Neurotoxic hippocampal lesions have no effect on odor span and little effect on odor recognition memory but produce significant impairments on spatial span recognition, and alternation. J Neurosci 2000;20:2964–77. Copyright 2000 by the Society for Neuroscience.
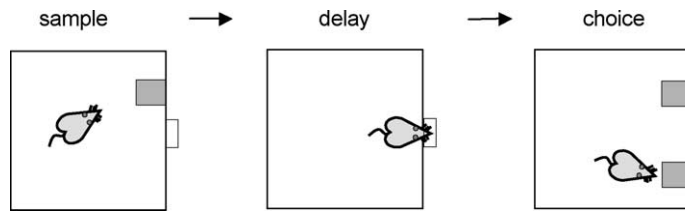
Fig. 7. A delayed non-matching to sample task in an operant chamber. The sample phase of the task consists of the presentation of a lever. During the ensuing delay, the lever is retracted and the rat must make a nose poke to a central food tray. Following the delay, the rat is presented with both levers, and reinforcement is obtained by a response to the lever that had not been presented during the sample phase of the trial.

reinforced when the current stimulus differed from the previous stimulus. In a review of operant DNMS tasks, Pontecorvo et al. [75] distinguish between this type of task—which they term a 'delayed comparison' task—and 'delayed response' tasks in which the rat can predict the correct response prior to the delay onset. [An advantage of the delayed comparison task, as will become evident below, is that postural mediation strategies are not possible.]

An example of the latter type of working memory task is the delayed non-matching to position task developed by Dunnett [76]. In this task, the lever to which the rat responds serves as the to-be-remembered stimulus (see Fig. 7). A trial begins with the insertion of one of two levers into the operant chamber. This serves as the sample. After the rat presses the lever, it is retracted and a delay ensues. During the delay the rat is required to make a nosepoke to a central location between the levers, and the first nosepoke after the scheduled delay results in the presentation of both levers. Responses to the non-sample lever are reinforced.

Rats performed at nearly 100% accuracy on this task with short delays, and performance dropped to 75–80% accuracy with a 16 s delay. The inclusion of a nose-poke response during the delay appeared to block obvious postural mediation of responses. (In a task without this delay requirement and with both levers present in the box throughout the trial, rats 'bridge' the delay with overt responses [80]).

Chudasama and Muir [81], however, explicitly examined the behaviour of rats during the delay period of this operant DNMS task. They video-taped rats during the delay period and had independent observers rate the behaviours that occurred. Strikingly, although rats were required to make a nosepoke response to a food magazine located between the levers during the delay, mediating responses were evident in the majority of delays. Rats tended to poke their nose into the side of the food magazine closest to one of the levers during the delay. Other strategies included orienting the head towards the to-be-correct lever while opening the food magazine with a paw. Raters who viewed only the behaviour of the rats during the delay period were accurately able to predict the response that the rat made in the choice phase of the task. As in the early experiments of Hunter, this postural mediation of a response during a delay period tempers any interpretation of performance as being solely in terms of memory.

Other versions of delayed matching to position task require the rat to nosepoke to a port located on the wall opposite to the levers (e.g. [77]). This separation of the delay response may make postural mediation during the delay more difficult, and delay dependent performance has been observed (see Fig. 8). However, a thorough study by Gutnikov et al. [78] raised the possibility that a motor 'turning' strategy might be used to bridge delays even when responses are required on the far wall of the apparatus. Gutnikov et al. trained rats on two versions of delayed matching task in an five-choice apparatus, and observed that rats were much more accurate when the to-be-remembered stimulus was on the periphery of the stimulus array, as opposed to the center. They hypothesised that rat kept a biased body position when it entered the food magazine on the far wall, and this enabled it to turn towards the correct stimulus during the choice phase of the task.

Summary. The promise of automated memory assessment in an operant non-matching to sample task has been qualified by evidence that postural mediation of the to-be-remembered response can occur. These are a concern in delayed response tasks, where the animal may know where it has to respond based on where the sample stimulus was presented. In delayed comparison tasks, postural mediation is not possible, and tasks such as the visual touchscreen procedure of Bussey et al. [79] may provide a useful tool for the valid assessment of working memory.
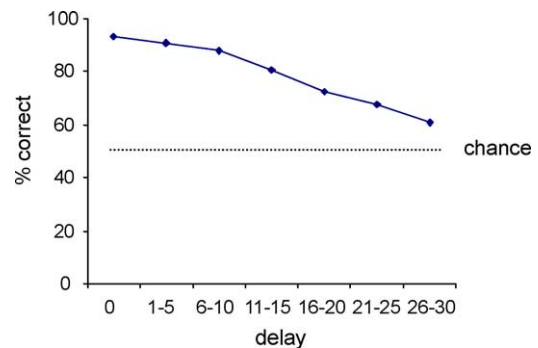

Fig. 8. Delay-dependent performance on an operant non-matching to sample task. Data adapted by Hampson RE, Jarrard LE, Deadwyler SA. Effects of ibotenate hippocampal and extrahippocampal destruction of delayed-match and -non-match-to sample behavior in rats. Journal of Neuroscience 1999;19:1492–507. Copyright 1999 by the Society for Neuroscience.

6. Conclusion

Working memory can be defined as a type of short term memory for stimuli or spatial locations that is typically used within a testing session, but not between testing sessions. This type of memory in animals was assessed in the delayed reaction studies of early researchers, and was formalised with the work of Olton and Honig in the 1970s. We have described a number of tasks that have been or are currently used for testing working memory. The most common of these are delayed alternation, the radial arm maze, spontaneous exploration, and DNMS paradigms.

Different challenges are associated with each of these paradigms. Maze tasks such as the T-maze or the radial arm maze can be labour intensive, and it is often difficult to specify the precise content of the memory that is used to solve the task. DNMS tasks have the advantage that the experimenter specifies the to-be-remembered stimuli. However, the encoding of experimenter-specified stimuli may also depend on the task requirements: active, reinforced encoding may recruit somewhat different neural systems than those tapped by spontaneous exploration. In addition, in rodent DNMS tasks, care must be taken that the rats are not responding to cues (e.g. odours) left by the experimenter over very short delays, or, more seriously, the bridging of delay periods by biases in posture or motor activity

In this regard the distinction between delayed reaction and delayed comparison tasks provided by Pontecorvo et al. [75] is a useful one. Delayed reaction tasks, in which the sample stimulus is sufficient to guide the animals choice response, leave open the possibility that the rat can bridge the delay period by postural orientation. Evidence for this behaviour was found in Hunter's initial delayed reaction paradigm, and even in recent operant non-matching to sample tasks. Delayed comparison tasks appear less susceptible to this confound, but these may not feasible for assessing spatial working memory based on the rats' tendency for spontaneous exploration.

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