

Nonrecurring-items delayed nonmatching-to-sample in rats: A new paradigm for testing nonspatial working memory

DAVE G. MUMBY, JOHN P. J. PINEL, and EMMA R. WOOD
University of British Columbia, Vancouver, British Columbia, Canada

Rats were trained on a nonrecurring-items delayed nonmatching-to-sample task, using a newly designed apparatus and a training protocol similar to that used in experiments on nonspatial working memory in humans and monkeys. On each trial, the rats were briefly presented with a sample object, which was presented again along with another object after a delay; the rats were rewarded with food if they chose the novel object. New stimuli were used on each trial. With delays of 4 sec between the sample and choice runs, the rats learned the task to 90% accuracy in less than 250 trials. When the delay was subsequently increased to 15, 60, 120, and 600 sec, the rats scored approximately 91%, 81%, 77%, and 57%, respectively. These results establish that rats are capable of excellent performance on a nonspatial working-memory task that is comparable to those commonly used in monkey models of amnesia, and they suggest that the nonrecurring-items delayed nonmatching-to-sample paradigm may prove valuable in modeling brain-damage-produced amnesia in rats.

Laboratory animals often perform poorly on tests of nonspatial working memory, despite performing well on similar tests of spatial working memory. For example, both monkeys (see, e.g., D'Amato, 1973; Nissen, Riesen, & Nowlis, 1938) and rats (see, e.g., Alexinsky & Chapouthier, 1978) perform poorly on conventional nonspatial delayed matching-to-sample and delayed nonmatching-to-sample tasks, although they perform well on comparable tasks that involve spatial stimuli (see, e.g., Nissen et al., 1938, monkeys; van Haaren & van Hest, 1989, rats). The poor performance of laboratory animals on nonspatial working-memory tasks has contributed to the view that the mnemonic abilities required to perform them have their phylogenetic origins in humans (see Iverson, 1976; Nissen et al., 1938).

It is now clear, however, that the poor performance of laboratory animals in many studies of nonspatial working memory does not reflect a lack of the prerequisite mnemonic abilities; it is rather an artifact of the methods typically used in these studies. Conventional nonspatial working-memory protocols involve a small set of test stimuli that are presented repeatedly, trial after trial. In the mid 1970s, it was shown by Gaffan (1974) and by Mishkin and Delacour (1975) that monkeys excel on a nonspatial working-memory task in which novel stimuli

are presented on each trial—the nonrecurring-items delayed nonmatching-to-sample task.

In the nonrecurring-items delayed nonmatching-to-sample task, each monkey is presented with a sample object, and following a retention interval during which the sample is hidden from view, the sample object is presented again, along with a novel item; the monkey is rewarded for selecting the novel item from this pair. Different objects are used on each trial. Monkeys learn this task quickly at retention intervals of only a few seconds, and once they have learned the nonmatching rule at short retention intervals, their performance is almost as good as that of humans at delays of up to several minutes (Squire, 1987).

The nonrecurring-items delayed nonmatching-to-sample paradigm has done more than establish that nonspatial working-memory abilities are not restricted to humans; it has become an integral part of the preeminent monkey models of human medial-temporal-lobe (Mishkin, 1978) and medial-diencephalic (Aggleton & Mishkin, 1983) amnesia.

We adapted the monkey nonrecurring-items delayed nonmatching-to-sample task for use with rats, and, using this adaptation, we demonstrated that rats, like monkeys and humans, are capable of excelling at an appropriately designed test of nonspatial working memory.

METHOD

Subjects

The subjects were 14 experimentally naive, male Long-Evans rats (Charles River, Quebec), 8 weeks old at the beginning of the experiment. They were housed individually with continuous access to water, and they were maintained on a 12:12-h light:dark cycle,

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with light offset at 11:00 a.m. Their body weights were reduced to 85% of ad-lib values by giving daily rations of laboratory chow, and their weights were maintained throughout the experiment at a level that was 85% of the typical weight of rats of the same age, sex, and strain that are maintained with continuous access to laboratory chow. Training commenced after the rats had been on the restricted feeding regimen for 14 days.

Apparatus

The apparatus, which was constructed of sheet aluminum (thickness = 0.127 cm), was a straight runway elevated 70 cm from the floor (see Figure 1). The apparatus was 60 cm long, 20 cm wide, and 40 cm high. Identical goal areas at each end of the runway were separated from the central starting area by two opaque guillotine doors, each located 30 cm from the nearest end wall. Each goal area had two recessed food wells, 3.5 cm in diameter and 2 cm deep. The food wells were separated by a short divider wall (9 × 9 cm), which protruded from the center of the end wall. The food wells were centered 5 cm from the divider wall, and 3 cm from the end wall. The sides of the goal areas were open to allow the experimenter to easily position stimulus objects over the food wells and quickly remove them. Food pellets were delivered to the food wells via funnels that were mounted on the outside of the apparatus and connected to the food wells with vinyl tubing.

We used a collection of over 350 test objects of various shapes, textures, and colors, similar to the "junk" objects used in the monkey nonrecurring-items delayed nonmatching-to-sample paradigm (see Mishkin & Delacour, 1975); each was large enough to cover a food well but small enough and light enough to be easily displaced by the rats. Some of these objects likely had distinctive odors, but

no objects with obvious scents were included. The food rewards were 45-mg pellets (Bio-Serv Inc., Frenchtown, NJ).

Procedure

All testing occurred during the dark phase of the light:dark cycle, between 14 and 21 h after the rat's most recent meal. The rats were not handled during a session once they had been placed in the apparatus, unless they urinated or defecated, in which case they were removed briefly so that the floor could be wiped clean. The rats were tested no more than once per day and no less than four times per week.

Pretraining. The pretraining phase consisted of six 20-min sessions. During the first two sessions, each rat was placed in the apparatus and allowed to explore. The guillotine doors remained open, and each of the four food wells was baited with three or four food pellets. The wells were rebaited each time that the pellets in all four wells had been consumed.

On the 3rd and 4th pretraining days, the rats were shaped to run back and forth between the goal areas by alternately baiting a single well at each end. Food appeared equally often in all four wells. The guillotine doors remained open during these two sessions.

We introduced the rats to the operation of the guillotine doors during the 5th and 6th pretraining sessions. At the start of both of these sessions, the food wells were unbaited, but when each rat approached a food well at one end to search for food, the experimenter lowered the door at the other end and baited one food well on the other side of it with a single pellet. When the rat approached to within about an inch of the lowered door, it was raised to provide the rat with access to the baited food well. As soon as the rat found the food, the far door was lowered, and one of the wells behind

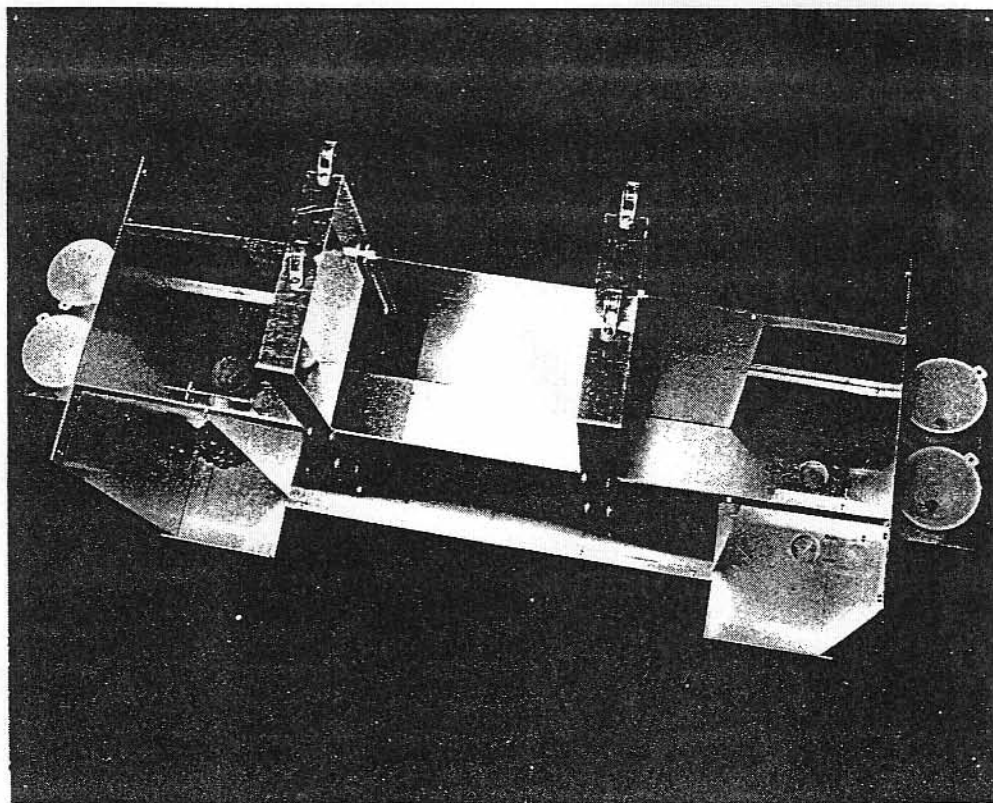


Figure 1. The nonrecurring-items delayed nonmatching-to-sample apparatus. (Photograph by Jack Wong.)

it was baited. This door was opened as soon as the rat approached it. This cycle was repeated throughout the fifth and sixth pretraining sessions, and by the end of Session 6, all 14 rats quickly approached the closed doors to gain access to the pellet on the other side.

Two-choice discrimination. Following the 6-day pretraining phase, each rat received four two-choice discrimination sessions, each comprising 25 trials. These two-choice discrimination sessions were designed to accomplish two goals: to teach the rats to gain access to food wells by displacing test objects from on top of them, and to eliminate the side preferences (i.e., preferences for either the right or left food wells) that were initially displayed by many of the rats (cf. Rothblat & Hayes, 1987).

For a particular subject, the same two objects served as the stimuli for all 100 of its two-choice discrimination trials. One of the objects was randomly designated the S+ (reward); the other object was designated the S- (no reward). To begin each session, the rat was placed in the center of the apparatus; one door was open, and one was closed. The S+ and S- were each placed over one of the food wells behind the closed door—the position of S+ (left or right) varied from trial to trial, according to an irregular but balanced pattern. Then the experimenter opened the door to expose the two objects. When the rat approached and displaced an object, the far door was lowered. If the rat displaced the S+, a food pellet was delivered to that food well; if the S- was displaced, no reward was provided. Correction was allowed during the first two-choice discrimination session; if the rat first chose S-, it was permitted to displace S+ to obtain a reward before the experimenter removed the objects. However, during the remaining two-choice discrimination sessions, correction was not allowed. The experimenter then positioned S+ and S- at the other end of the apparatus in preparation for the next trial. The duration of the intertrial interval varied, but it was typically 15-20 sec.

Acquisition of nonrecurring-items delayed nonmatching-to-sample. In preparation for training the subjects to perform the nonrecurring-items delayed nonmatching-to-sample task, 350 test objects were divided randomly into seven sets of 50 objects each; the objects used for the two-choice discrimination were included. A different set was used on each consecutive 25-trial session (i.e., a particular set was used on every seventh session). Different pairs of objects were used for each of the 25 trials within a session; one was randomly designated the sample, and the other the novel object. The pairs were randomized every time a set was reused (i.e., identical pairs recurred only by chance).

To begin a session, the rat was placed in the apparatus with the doors raised; approximately 1 min later, the doors were lowered to enclose the rat in the central starting area. Before each trial, a single food pellet was placed in a food well and the sample object was placed over it. The location of the pellet and the sample object varied according to an irregular, but balanced, schedule; they appeared at each of the four food wells with equal probability. Once the sample object was positioned, the novel object was placed over one of the food wells at the other end of the apparatus; its position, left or right, varied according to an irregular, but balanced, schedule.

To begin a trial, the experimenter raised the door to allow access to the sample object, which the rat approached and displaced from the food well. While the rat ate the food pellet, the experimenter removed the sample object and positioned it over the vacant food well at the other end of the apparatus. The other door was then raised, and the rat approached and displaced either the sample object or the novel object. A food pellet was delivered to the exposed food well if the novel object was displaced; no pellet was delivered if the sample object was displaced—rats were considered to have made a choice only if they moved an object enough to expose the food well. The rats were permitted to make corrections during the first session, but not thereafter. As the rat started to eat the pellet, the experimenter immediately removed the objects and lowered the door farthest from the rat. When the rat

finished eating, it reentered the central starting area and the other door was lowered behind it to enclose it in the central starting area. The latency of the rat to return to the start area determined the intertrial interval (i.e., the amount of time between the rat's displacing an object on the choice run of a trial and the experimenter's raising the door to allow access to the sample object on the next trial); the next trial began as soon as the rat had been enclosed in the starting area and the new sample and novel objects were positioned. Most intertrial intervals were 30-40 sec in duration. If a particular rat was consistently slow to return to the starting area, it was occasionally rewarded with a food pellet as it entered the starting area.

The time between the removal of the sample object and the raising of the second door to provide access to the sample and novel objects (i.e., the retention interval, or delay) was approximately 4 sec during the initial training sessions. For a particular subject, training continued at this 4-sec retention interval until it reached the criterion of at least 21 out of 25 correct choices on two consecutive sessions, whereupon the retention interval was increased to 15 sec. The retention interval was subsequently increased to 30, 60, and finally, 120 sec, whenever a rat either reattained the criterion (two consecutive sessions of at least 21 correct) or had eight sessions at a particular delay without achieving criterion. Each rat received four sessions at a 600-sec delay interval after training at the 120-sec delay was completed.

Measuring the retention function. The final phase of testing was designed to define each rat's retention function. This phase consisted of five mixed-delay nonrecurring-items delayed nonmatching-to-sample sessions, each of which consisted of 25 trials. In each of these sessions, five trials were conducted at each of the following delays; 4, 15, 60, 120, and 600 sec. These delays appeared in the following order in each session: 4, 15, 60, 120, 600, 600, 120, 60, 15, 4, 4, 15 sec, and so on.

RESULTS

All 14 rats learned the nonrecurring-items delayed nonmatching-to-sample task, and statistically significant levels of performance were achieved at all delays.

Two-Choice Discrimination

Although there was some initial hesitation, all of the rats quickly learned to displace the objects from the food wells. On the first two-choice discrimination session, the mean number of correct trials was 58% (ranging from 44% to 76%, $SE = 2.67\%$), and on the fourth (i.e., final) session, the mean was 91% (ranging from 72% to 100%, $SE = 2.08\%$).

Acquisition of Nonrecurring-items Delayed Nonmatching-to-Sample

Figure 2 illustrates the performance at each delay of the nonrecurring-items delayed nonmatching-to-sample task during the acquisition phase. Illustrated are the mean levels of performance on the 1st and last sessions at each delay. Performance on the 1st training session at the 4-sec delay was significantly above chance [$M = 59\%$, $t(13) = 4.14$, $p < .005$, two-tailed], as was performance on all subsequent sessions. At the 4-sec delay, 13 of the 14 rats reached criterion within 16 sessions, not including the final 2 criterion sessions ($M = 9.4$ sessions, or 235 trials). The 1 rat that did not achieve criterion scored as high as 88% on single sessions, but was inconsistent; this

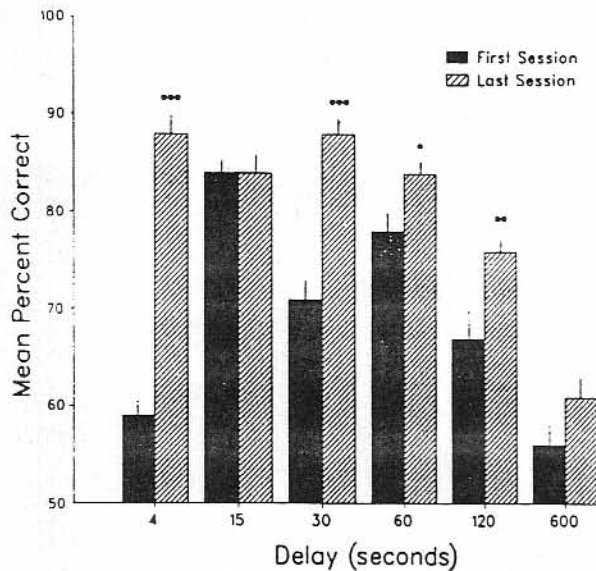


Figure 2. Mean percent correct on the first and last sessions at each delay during acquisition training on nonrecurring-items delayed nonmatching-to-sample. Performance improved between the first session and the last session at most delays (* $p < .05$, ** $p < .01$, *** $p < .001$).

rat was switched to the 15-sec delay condition after 20 sessions.

As is illustrated in Figure 2, when rats were switched to delays longer than 15 sec, their performance declined initially, and then it improved over sessions at the new delay. The numbers of rats reattaining criterion within the maximum of eight sessions at the 15-, 30-, 60-, and 120-sec delays were 8, 12, 9, and 2, respectively. No rats achieved criterion within the four sessions that were administered at the 600-sec delay.

Retention Function

Figure 3 illustrates the mean retention functions of the 14 rats, calculated from their performance on the five mixed-delay nonrecurring-items delayed nonmatching-to-

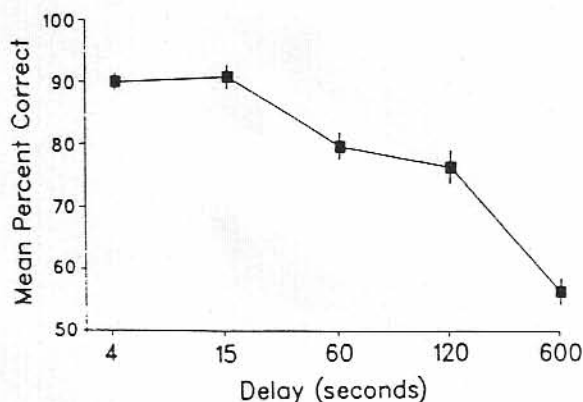


Figure 3. Mean percent correct on mixed-delay nonrecurring-items delayed nonmatching-to-sample sessions. Retention was statistically significant at each delay (all $ps < .01$).

sample sessions. The results of the repeated measures analysis of variance indicated that performance declined significantly as the retention interval increased [$F(4,52) = 75.72, p < .001$]. However, performance at the 600-sec delay was still significantly better than chance [$t(13) = 2.77, p < .05$, two-tailed]. Performance at all delays was stable over the five sessions.

Although the direction (left or right) of the errors was not recorded, several rats seemed to make most of their errors when the novel object was on a particular side, especially on trials with long retention intervals.

DISCUSSION

In this experiment, rats' performance on the nonrecurring-items delayed nonmatching-to-sample task was comparable to that commonly reported for monkeys in terms of both the rate of acquisition of the nonmatching rule and the accuracy of their choices at various retention intervals. The rats required a mean of 235 trials to achieve the initial criterion of 84% on two consecutive sessions; rhesus monkeys (Mishkin & Delacour, 1975), cynomolgus monkeys (Aggleton & Mishkin, 1983), and squirrel monkeys (Overman, McLain, Ormsby, & Brooks, 1983) required means of 90, 150, and 785 trials, respectively, to achieve a slightly more stringent criterion (e.g., at least 90% correct on two consecutive sessions, or at least 90 correct on 100 consecutive trials). During the final mixed-delay test sessions, the rats in the present study averaged 90%, 91%, 81%, and 77% at retention intervals of 4, 15, 60, and 120 sec, respectively. This level of asymptotic performance compares favorably to the asymptotic levels observed in monkeys at comparable retention intervals; asymptotic scores of monkeys typically range between 90% and 100% at the shortest intervals and between 85% and 95% at 120 sec (see, e.g., Aggleton & Mishkin, 1983; Murray & Mishkin, 1986). At 600 sec, the rats scored 57% correct; although this level of performance is significantly above chance, it is considerably lower than the 80% reported for monkeys (Zola-Morgan, Squire, & Amaral, 1989).

On Day 1 of nonrecurring-items delayed nonmatching-to-sample training, the rats in the present study scored 59%, which was significantly above chance. This better-than-chance Session 1 performance presumably reflected their spontaneous tendency to approach novel stimuli, and it confirmed previous reports of better-than-chance performance on initial nonrecurring-items delayed nonmatching-to-sample trials by both monkeys (Mishkin & Delacour, 1975) and rats (Aggleton, 1985; Rothblat & Hayes, 1987). The acquisition of the nonmatching rule was reflected in the significant improvement in the rats' performance as the training sessions progressed. The initial drop in performance during the acquisition phase that typically occurred when the delay was lengthened and the subsequent improvement in performance that occurred over sessions at each delay suggest that rats either gradually learned to avoid distraction for increasing periods of time, or else became more efficient at encoding features

of the sample object—or both. It is likely that the consistency of performance at each delay over the five mixed-delays sessions was at least partly due to the extensive acquisition training.

The positional biases that appeared on trials with longer delays make the interpretation of accuracy scores less straightforward. We presume that positional biases are a by-product of poor retention, that the rats tended to resort to a positional strategy whenever they were unable to perform on the basis of their memory of relevant nonspatial cues. However, it is also possible that causal effects flowed in the other direction—that agitated behavior associated with the longer delays interfered with the rats' memory of the sample objects. Agitated behavior has been reported in rats during long delays in a delayed nonmatching-to-sample task (Rothblat & Hayes, 1987), and it was also observed on some occasions in the present experiment. Side preferences might result from increased agitation from having to endure long delays, and such an effect could account in part for the drop in choice accuracy.

The experimental design ensured that consistently correct responding depended on recognition of the sample objects and application of the nonmatching principle, but the design did not ensure that the rats responded correctly on the basis of their memory for the visual properties of the samples. The rats may have responded correctly on the basis of their memory for the olfactory or tactual properties of the sample, or they may have circumvented the mnemonic demands of the task by marking the sample object during the sample runs and then performing an olfactory discrimination on the choice runs. However, the following observations suggested that the rats were making correct choices primarily on the basis of their memory of the nonspatial visual properties of the sample objects: (1) Although rats sometimes contacted one of the objects on the choice run with their vibrissae, and then, without moving it, turned and displaced the other object, such occurrences were rare. (2) On several trials, the rats veered toward the correct object while running down the runway, still several centimeters away. (3) To ensure that rats were not marking the sample, a separate series of control tests was conducted prior to the present experiment; on these trials, two identical objects were used as the sample on each trial—one on the sample run of the trial and the other on the choice run of the same trial. Performance on these control trials was comparable to that of the rats in the present experiment that were tested at similar delays; the two rats tested scored 90%, 88%, 82%, and 78% at delays of 20, 40, 80, and 120 sec, respectively.

The apparatus used in the present experiment has two notable features that may account for the rats' excellent performance. First, the two separate goal areas permitted the experimenter to position the sample and novel objects before the start of a trial, so that initial training could be conducted at very short retention intervals (i.e., 4 sec). Second, the presence of a central starting area eliminated

the need to handle the rats during the retention intervals (cf. Rothblat & Hayes, 1987); distraction during retention intervals has been shown to disrupt the nonrecurring-items delayed nonmatching-to-sample performance of monkeys (Zola-Morgan & Squire, 1985; Zola-Morgan et al., 1989).

The present study is not the first to demonstrate high levels of nonspatial working memory in rats; Aggleton (1985) recorded scores only slightly lower than those of the present study on a Y-maze version of nonrecurring-items delayed nonmatching-to-sample. However, it is the first to demonstrate impressive nonspatial working memory in a rat paradigm expressly designed to mimic the standard monkey delayed nonmatching-to-sample paradigm. Accordingly, it is now possible to make direct comparisons between the nonspatial working memories of rats and monkeys.

As an efficient method for testing nonrecurring-items delayed nonmatching-to-sample in rats, the present paradigm has potential value beyond facilitating the comparison of monkey and rat memory. Nonrecurring-items delayed nonmatching-to-sample is used in monkey models of brain-damage-induced amnesia (see, e.g., Mishkin, 1978; Zola-Morgan & Squire, 1985), and the present procedure might similarly lend itself to use in a rat model of amnesia. Such a rat model of amnesia would be accessible to many researchers who are interested in animal models of amnesia but lack primate facilities, and it would make it more practical to conduct the large-scale parametric studies needed to resolve some of the central theoretical issues in this field. In recent years, studies of monkeys have begun to enable the delineation of brain structures whose damage results in recognition memory deficits (e.g., Murray & Mishkin, 1986; Zola-Morgan & Squire, 1985; Zola-Morgan et al., 1989); we have begun a series of experiments to examine the effects of similar lesions on nonrecurring-items delayed nonmatching-to-sample in rats (Mumby, Pinel, & Wood, 1989; Wood, Mumby, Pinel, & Phillips, 1989).

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