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Delayed matching-to-position performance in C57BL/6N mice

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Abstract

Delayed matching-to-sample is one of the most frequently employed behavioral tasks for assessing spatial working memory in animals. Although the advantages of the task have been widely acknowledged and it is used in the study of a variety of species, its application to mice has been rare. In the present study, we reported the efficacy of a delayed matching-to-position task in C57BL mice lever-pressing in an operant-conditioning chamber. Each trial started with the press of a back lever, followed by the presentation of either a left or right front lever. When the ratio requirement for presses to the front lever (sample) was met, a delay interval started. Delay interval continued until the mice made the first response after the elapse of the programmed delay interval. This was followed by the presentation of a choice of left or right front levers. The choice of the same front lever as the sample was reinforced, whereas the other was not. The proportion of correct choices showed a delay-dependent decrement. A higher ratio of response requirement to the sample resulted in increased accuracy, but the duration of the intertrial interval had no effect. Preceding trials also influenced response accuracy, indicating proactive interference. Overall, the results replicated the effects of parametric manipulations reported in other species, and thus, our findings validate the efficacy of the task for assessing spatial working memory in laboratory mice.

Keywords: delayed matching-to-position; spatial learning; working memory; operant; C57BL/6N; mice
1. Introduction

Delayed matching-to-sample (DMTS) tasks using operant-conditioning chambers have been a powerful means by which to study animal working memory (Blough, 1959; D'Amato, 1973; D'Amato and Worsham, 1974; White, 1985), but surprisingly, few studies in laboratory mice have been carried out in operant-conditioning chambers. Instead, numerous versions of maze tasks have been developed to examine genetic and physiological factors involved in spatial reference and working memory in various strains of mice, and many studies in mice have been carried out with maze procedures (Brown and Wong, 2007; Deacon and Rawlins, 2006; Hodges, 1996; Yoshida et al., 2001).

When examining spatial working memory in mice, T-maze alternation (Deacon and Rawlins, 2006) and delayed matching (nonmatching)-to-place (Morris and Frey, 1997; Steele and Morris, 1999; Wietrzych et al., 2005) have often been used. In the former procedure, entering one of two arms is reinforced on alternating trials. The number of entries ascribed to correct and incorrect arms are behavioral measures. In the latter procedure, the animal is first released into a T-maze in which one of the two arms is blocked. This is the acquisition phase. After a short delay following the animal’s arrival at the reinforcer placed at the end of the open arm, the animal is placed back at the start position and is then released into the maze with both arms opened. A choice is reinforced and considered correct if the animal enters the arm visited (matching) or not visited (nonmatching) during the acquisition phase. Although delayed responding paradigms using maze procedures allowed us to examine working memory in animals, the use of a relatively novel paradigm such as those in operant-conditioning chambers extend the generality of the findings in maze paradigms with the precise control of retention and intertrial intervals (ITIs) as well as incorporating new measurements of behaviors such as lever pressings.

Dunnett and colleagues (Dunnett, 1985; Dunnett and Martel, 1990; Dunnett et al., 1988, 1990) developed a lever-pressing delayed matching-to-position (DMTP) protocol for examining spatial working memory in rats by modifying the delayed conditional discrimination task (Herremans et al., 1994; Wallace et al., 1980). At the beginning of the trial, one of the front retractable levers, left or right, was extended into the chamber. When rats
responded by pressing the lever, the lever retracted, and the delay interval clock was started. During the delay, rats made either nose poke responses at the panel (e.g., Dunnett, 1985) or performed rear-lever pressings (e.g., Bailey and Mair, 2005; Burk and Mair, 1998, 2001). The first such response after the end of the programmed delay caused both levers to be extended. The choice of the same lever as the sample was reinforced, whereas the choice of the other was not. Memory of the lever location decayed over lengthening delay intervals, but it did not drop to chance even with delays of around 30 s in rats.

Although the lever-pressing DMTP and other versions of delayed lever-pressing paradigms (e.g., Heise, 1984; Heise et al., 1976; Pontecorvo, 1983) are fundamentally similar to maze paradigms as a means of testing spatial working memory, lever-pressing procedures have numerous analytic advantages over maze procedures in terms of more precise control of timing and behaviors.

Two notable examples are the manipulations of ITI duration (Dunnett and Martel, 1990) and the fixed-ratio requirement for sample responding (Burk and Mair, 1998). Dunnett and Martel (1990) revealed that matching accuracy was higher in the trials in which the sample lever was same as that used in the previous trial whereas matching accuracy decreased in the trials in which the sample lever was different from that used in the previous trial. These results suggest that proactive interference is one of the potential processes influencing the successful retrieval of sample memory. The authors further revealed that such interference diminishes as the ITI increases (Dunnett and Martel, 1990). Burk and Mair (1998) showed that matching accuracy was higher when the response count requirement for the sample lever was larger, independent of delay interval duration, suggesting that the sample response requirement affects the discriminability of the sample location, but not the forgetting rate.

Thus, the purposes of the present study were to validate the DMTP task (adopted from the procedure used by Mair and colleagues, i.e., Bailey and Mair, 2005; Burk and Mair, 1998, 2001) as a measure of the construct of working memory and to demonstrate the analytical power of this method by characterizing the effects of a number of parametric manipulations on working memory in laboratory mice. Lever-pressing DMTP tasks have already been used
to examine the effects of genetic or pharmacological manipulations on laboratory mice (Bernardo et al., 2007; Escher and Mittleman, 2004; Estape and Steckler, 2001; Krueger et al., 2006; Martin et al., 2004; Nordquist et al., 2008; Woolly and Ballard, 2005). However, previous authors were less interested in examining how the effects parametric manipulations influence the discriminability and forgetting rate on DMTP performance in mice. The present study, then, examined the effects of changing both the fixed ratio (FR) requirement for sample responding and the duration of the ITI. In addition, proactive interference was examined, since it is one of the most pervasive parameters in the paradigm.

2. Method

2.1. Subjects

Subjects were five male C57BL/6N Crj mice obtained from CLEA Japan, Inc. (Tokyo, Japan) and were 12 weeks old when the experiment began. Although naïve to lever-pressing tasks, all mice had experienced a paw preference test and an open-field maze task prior to the present experiment. The mice were housed in groups of three and two in cages (29 cm long × 19 cm wide × 13 cm high) and were kept on a 12-h light/dark schedule. Training was carried out during the dark phase. Mice were kept at or above 85% of their free-feeding weight, which was maintained by supplementary feeding in addition to the food reinforcers in daily testing sessions. Water was freely available in the housing cages. The experiment reported here was conducted in accordance with the guidelines published by the Japan Society for Animal Psychology and was approved by the Animal Care and Use Committee of Keio University (No. 08007).

2.2. Apparatus

An operant-conditioning chamber (ENV-307A; Med Associates, Georgia, VT) with internal dimensions of 21.6 cm long × 17.8 cm wide × 12.7 cm high was used. The chamber was housed in a sound-attenuating box in a test room and was equipped with three retractable levers (ENV-312M): two on the front wall and one on the back. A 1.0-A house light was positioned above the back lever, and two 1.0-A lights, which were not used in the present experiment,
were positioned above the front levers. A food well was positioned in the center of the front panel into which a 25-mg food pellet (Obara Medical, Tokyo, Japan) was delivered by a dispenser (ENV-203-20) to reinforce correct responses. Masking noise was provided by 75-db white noise throughout experimental sessions. A Pentium IV computer (Dell Optiplex, Round Rock, TX) situated outside the testing room controlled and recorded all experimental events and responses via an interface (Med Associates).

2.3. Procedure
2.3.1. Initial training

Mice were first given magazine training, and then lever pressing to the left and right front levers and to the back lever (one lever at a time) was manually shaped, during which time a single lever press provided a 25-mg food pellet. After the acquisition of front and back lever pressing, two-press shuttle training was introduced. In the two-press shuttle training, a food pellet was given after completion of a sequence of presses on levers that were extended into the chamber. Levers were extended one at a time and retracted (and the next lever was extended) after a single lever press. Each sequence started with the back lever extending, followed by one of the two front levers (randomly selected for each trial). The second lever press was reinforced by a 25-mg pellet. Following acquisition of the two-press shuttle training, the response sequence was extended to four-press shuttle training in which the sequence started with the back lever extending, followed by one of the two front levers, then the back lever again, and finally, the same front lever that was extended previously in the sequence. The fourth lever press was reinforced by a 25-mg pellet. Mice continued on the training regimen until all the mice reliably completed 40 trials in a session for five successive days.

2.3.2. DMTP training

DMTP training was the same as in the four-press shuttle training, except for the fourth extension in the sequence. Mice were required to make a sequence of responses when levers were extended into the chamber. The first three extensions were exactly the same as in the four-press shuttle training: the
back lever, one of the two front levers (the sample lever for that trial), and the back lever again. In the fourth extension of the sequence, unlike the four-press shuttle task, both front levers were extended. A press on the sample lever (a correct response) was reinforced. A press on the other lever (an incorrect response) ended the trial without reinforcement. When the subject made an incorrect response, the same trial was repeated a maximum of two trials in a row (correction trials). Because one mouse exhibited strong position bias (i.e., it pressed the front lever on one side only throughout the sessions), four-press shuttle training with only the front lever not chosen by the mouse was conducted for four sessions in an attempt to eliminate the position bias. These forced-choice sessions were excluded from the subsequent analysis. The ITI was 3 s, during which the house light was turned off. Otherwise, the house light was lit throughout the experimental sessions. Each daily session consisted of 40 trials, except for correction trials. When mice did not complete 40 trials after 45 min, the session was terminated. Training was conducted six days a week until 30 sessions had been completed, excluding forced choice sessions.

2.3.3. Test 1

After 30 sessions of DMTP training, the DMTP procedure was modified in two ways. First, the sample lever press count requirement was increased to FR2 or FR5, randomly intermixed without replacement on a trial-by-trial basis. Second, the duration of the back lever presentation following the completion of sample lever responses was also lengthened by imposing delay intervals of 1, 3, 5, 7, or 9 s. Delay intervals were also randomly intermixed without replacement on a trial-by-trial basis. In order to extenuate behavioral mediating strategies such as staying in front of the correct lever during delay intervals, back lever pressing was imposed in the present experiment. Thus, mice repetitively pressed the back lever during the delay interval, and the first press after the elapse of the given delay interval resulted in retracting the back lever and extending both front levers for the choice response. Chudasama and Muir (1997) revealed that nose poke response requirements to the food magazine located between the two choice levers during the delay interval did not completely eliminate potential mediating behaviors such as rats' orienting
towards the correct lever. Furthermore, they revealed that such mediating behaviors occurred more frequently at shorter than longer delay intervals, implying effect of forgetting confounded by behavioral strategies adapted by rats. In the present study, we recorded the number of back lever presses and the time between sample and comparison responses in order to examine whether the frequency of mice’s use of potential mediating behaviors such as waiting in front of the correct lever differed among delay intervals. Each daily session consisted of 40 trials (two sample lever positions × 5 delay intervals × 2 sample FRs × 2 cycles), excluding correction trials; however, the session was terminated after 45 min regardless of the number of completed trials. Because one mouse exhibited position bias during the test, it received four sessions of the forced choice lever training with the ignored lever. Test 1 consisted of 20 sessions, excluding the forced choice sessions.

2.3.4. Test 2

Following Test 1, the DMTP procedure was further modified in three ways. First, the sample lever press count requirement was fixed at FR2. Second, the ITI was set to either 3 s or 13 s, randomly intermixed without replacement on a trial-by-trial basis. Third, the five values of programmed delay intervals were changed to 1, 5, 7, 9, and 13 s. Each daily session consisted of 40 trials, excluding correction trials, but was terminated after 45 min regardless of the number of completed trials. Test 2 again consisted of 20 sessions.

2.3.5. Test 3

Following Test 2, the DMTP procedure was modified so that the parameters were the same as in Test 1 except for the five values of programmed delay intervals. The five values of programmed delay intervals were changed to 1, 5, 10, 15, and 20 s. Each daily session consisted of 40 trials, excluding correction trials, but was terminated after 45 min regardless of the number of completed trials. Test 3 again consisted of 20 sessions.

3. Results

3.1. Acquisition of the delayed matching to position task
Performance was first assessed by calculating the means of the proportion correct scores, where proportion-correct is calculated as the number of correct responses divided by the total number of responses in a session, excluding correction trials. Figure 1a shows mean proportion correct values for five mice in the first 30 sessions. Mice performed at chance in the first 10 sessions but gradually improved their performance as training proceeded. In accordance with the improvement in response accuracy, the number of correction trials per session decreased as training proceeded, and its asymptotic level was around 10 correction trials per session (Figure 1b). Only the mouse that exhibited a strong position bias towards the right front lever received forced choice correction in order to eliminate the bias. Including this subject, all mice acquired the task and achieved a proportion correct score of 0.80 or above by session 30.

3.2. Test 1

After the introduction of delay intervals, sessions were divided into three blocks of 20 sessions (Tests 1, 2, and 3, respectively), and data were separately pooled for each block. In the first block of 20 sessions, the FR requirement for the sample lever was varied on a trial-by-trial basis. In Figure 2, the means of proportion correct averaged over 20 sessions for two FR requirements (FR2 and FR5) are plotted as functions of delay interval. Response accuracy decreased with increasing delay interval. When the response requirement for the sample lever was varied, accuracy was higher with FR5 than with FR2. Two-way repeated measures of ANOVA (sample FR and delay interval) revealed significant main effects of FR requirement for sample, $F_{1,19} = 5.46, P < 0.05$, and delay interval, $F_{4,76} = 30.44, P < 0.001$. The interaction between the two was not significant: $F_{4,76} = 0.54$.

When interpreting the effect of delay interval, one may be concerned that the mouse might physically “bridge” the delay interval by, for example, positioning itself near the correct lever. In order to extenuate such mediating strategies during delay intervals, back lever pressing was imposed in the present experiment. The middle row of Figure 2 shows the means of response times (time between the retraction of the sample lever and subject’s comparison.
choice) plotted as functions of delay interval. Mouse response times linearly increased as the time of the imposed delay interval increased, and a very small individual difference was observed. Engagement of the mice in back lever pressing during the delay intervals was further supported by the number of back lever responses. The bottom row of Figure 2 shows the arithmetic means of the number of back lever responses averaged over subjects for two sample lever FR values plotted as functions of delay interval. The number of back lever responses linearly increased as the delay interval values increased. Response requirement for the sample lever had no effect on response time and number of back lever responses.

3.3. Test 2

In this block of sessions, our primary interest was to examine whether response accuracy is higher when trials were spaced out with longer ITIs. Proportion correct averaged over 20 sessions for two ITIs (3 and 13 s), response time and the number of back lever presses are plotted as functions of delay interval in the middle column of Figure 2. Proportion correct decreased with increasing delay interval in a manner similar to Test 1. However, no apparent difference of performance was observed between the two different ITIs. Two-way repeated measures of ANOVA revealed a significant main effect of delay interval, $F_{4,76} = 16.61, P < 0.001$, but no other effects were significant (ITI: $F_{1,19} = 2.53$; ITI × delay interval: $F_{4,76} = 0.38$).

3.4. Test 3

In this block of sessions, we re-examined the effect of the FR requirement for sample when extending the upper range of the delay interval to 20 s (the right column of Figure 2). In general, results replicated Test 1, showing a delay-dependent decrement of accuracy and better accuracy with a higher FR response requirement for the sample stimulus. Two-way repeated measures of ANOVA revealed a significant main effect of delay interval, $F_{4,76} = 32.55, P < 0.001$, and a main effect for FR requirement that barely missed significance: $F_{1,19} = 4.17, P = 0.055$. An interaction between FR requirement and delay interval was also significant: $F_{4,76} = 3.69, P < 0.01$. Simple effects of FR requirement at
delay intervals of 5 s and 15 s were significant (5 s: $F_{1,19} = 10.95$, $P < 0.01$; 15 s: $F_{1,19} = 5.41$, $P < 0.05$).

3.5. Intertrial proactive interference

As described previously, errors on trial N should be more likely when the opposite side lever was selected as a sample on trial N-1 due to intertrial proactive interference. Figure 3 shows means of proportion correct when the sample lever on the present trial was the same or different in the preceding trial (the data was pooled only when response on trial N-1 was correct in order to eliminate artifacts caused by correction trials). Indeed, performance was better in the same lever than in the different lever trials, indicating a proactive interference effect, and the trend was consistent across the three test blocks. Two-way repeated measures of ANOVA revealed a significant main effect of previous trial: $F_{1,19} = 22.38$, $P < 0.001$. No other effects were significant (session block: $F_{2,38} = 2.10$; previous trial × session block: $F_{2,38} = 0.16$).

3.6. Response time

Burk and Mair (1998) examined the relation between accuracy and response time in rats during the DMTP task and found short responses tended to be more accurate. We therefore compared response time (from the onset of availability of the comparison levers) between correct and incorrect trials. Response times were log transformed to meet the assumption of normality prior to statistical analysis. Figure 4 shows means of response time in correct and incorrect trials in the three test blocks. In all three blocks, response time tended to be faster in correct than in incorrect trials. Two-way repeated measures of ANOVA showed a significant main effect of trial outcome: $F_{1,19} = 64.48$, $P < 0.001$. A significant interaction was also observed between trial outcome and session block: $F_{2,38} = 4.04$, $P < 0.05$. Orthogonal contrast analysis revealed that contrast-contrast interactions of session blocks 1 and 2 ($P < 0.001$) and session blocks 2 and 3 ($P < 0.05$) were significant. The effect of session block was not significant: $F_{2,38} = 0.34$.

4. Discussion
The results of the present study illustrate the validity of the lever-pressing delayed matching-to-position task as a means of quantifying working memory and characterize the effects of a number of parametric manipulations in testing laboratory mice. Validation of the technique was demonstrated first by verifying a delay-dependent decrement of response accuracy, and second by demonstrating the effects of FR requirement to the sample on DMTP performance. Third, it was shown that intertrial proactive interference effects hamper DMTP performance. These results are also compatible in many respects with previous studies in animal delayed matching- and nonmatching-to-sample tasks (Edhouse and White, 1998; White, 2001).

The effects of FR requirement to the sample (Figure 2a and 2c) were persistent throughout the experimental sessions, and the proportions of correct responses were higher in trials with larger rather than smaller FR requirements, indicating that the discriminability of the sample was enhanced by imposing a requirement for a higher number of responses to it (Burk and Mair, 1998, 2001; Cohen et al., 1976; Roberts, 1972; White, 1985). Although the FR requirement influenced response accuracy, it had no effect on behavior during delay intervals, such as time between sample and comparison response (Figure 2d and 2e) or the frequency of back lever presses (Figure 2g and 2i), suggesting that the discriminability of the lever position could be systematically and selectively manipulated by the FR requirements in the DMTP procedure.

Unlike the effects of the FR requirement, little effect of ITI on the three behavioral measures was observed in the present experiment (Figure 2b, e, and h). In previous studies with pigeons (White, 1985) and rats (Bushnell, 1988; Dunnett and Martel, 1990), the proportion of correct responses was higher in trials following longer ITIs. Such effects were derived at least partially from intertrial proactive interference effects (Dunnett and Martel, 1990). There may be two possible explanations for the failure to find an ITI effect in the present study. First, unlike rats, mice either in general or in the C57BL strain may be insensitive to this parameter for unknown reasons. It is well known that the strategies that animals apply to a particular task may differ even between closely related species, depending on their ecological niche (Lea et al., 2006). One may consider that such a species-specific rationale is inconclusive. However, two
values of ITIs in the present study (3 s and 13 s) were sufficiently similar to the
values for which Dunnett and Martel found a marked difference of accuracy in
rats, implying that mice, unlike rats, are insensitive to this parameter.

Another possibility could involve the discriminability of the ITI from the
delay interval (Cohen and Njegov, 1999; Zentall, 1997). In the present study,
an ITI was always signaled by turning off the house light as well as by the
retraction of all three levers. Perhaps the combination of the blackout and the
retraction of the levers resulted in better discrimination between the ITI and the
within-trial contexts, such as the delay interval, thereby reducing or eliminating
the ITI effect.

Although an ITI effect was not observed, proactive interference of the
sample was observed between trials. Proactive interference could be examined
by comparing trials in which the sample in trial N was the same or different from
that in the preceding trial (N-1). The proportions of correct responses were lower
in the different-sample trials compared with the same-sample trials (Figure 3),
indicating a proactive interference effect in a similar manner as found in rats
(Burk and Mair, 1998; Dunnett and Martel, 1990). Neither the FR requirement
parameter nor ITI cancelled out the effects of proactive interference. The effects
were also not negligible even after extensive training (each mouse received
about 3,600 trials in 90 sessions, except for corrections), suggesting that
proactive interference is one of the primary mechanisms of matching errors in
the DMTP task.

Finally, we examined response times during the DMTP task. There was
a tendency for responses, with short response times being more accurate, and
the finding was consistent with that in the other taxa (birds: Goto and Watanabe,
2009; primates: Hampton and Hampstead, 2006). Furthermore, the difference of
response times between correct and incorrect trials was greater when the
response requirement for sample was not manipulated (i.e. Test 2) than when it
was manipulated (i.e. Tests 1 and 3). These results imply that the simple
speed/accuracy tradeoffs do not fully account for the patterns of response times
in the DMTP task and experimental variables such as the response requirement
for sample should also be taken account for the variations in response times.

In conclusion, the present study validates the efficacy of the operant
DMTP task as a means of assessing spatial working memory in laboratory mice. Parametric manipulations, such as of delay interval and FR requirement to the sample lever, permit quantitative description of various aspects of memory processes. The results of the present study also support the idea that proactive interference was one of the major mechanisms causing errors in the DMTP task, and thus, susceptibility to memory interference can also be examined with this paradigm. The lever-pressing DMTP task therefore has numerous advantages over the currently pervasive maze tasks in assessing spatial working memory in laboratory mice. This task should facilitate understanding of the effects of lesions, genetics, and pharmacology on spatial working memory in this species.

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References


Figure legends

Figure 1. Acquisition of the delayed matching-to-position task. (a) Proportion correct was calculated as the number of correct trials divided by total trials, excluding correction procedures. (b) Mice repeatedly received the same trials when making errors. Correction trials were not given more than twice for each trial. Error bars indicate standard error of the mean (SEM).

Figure 2. Delayed matching-to-position performance at different delay intervals. Proportion correct (a, b, c), response time (time between sample and comparison responses) (d, e, f), and number of back lever responses during delay intervals (g, h, i) are shown separately in Tests 1, 2, and 3. Error bars indicate the 95% confidence intervals for a repeated measures design (Loftus & Masson, 1994). FR = fixed ratio; ITI = intertrial intervals.

Figure 3. Proactive interference effect. Proportion correct was shown separately for trials in which the sample on the previous trial was on the same (same trials) or the opposite side (different trials) to the sample on the current trial. Error bars indicate the 95% confidence intervals for a repeated measures design (Loftus & Masson, 1994).

Figure 4. Response time was shown separately for correct and incorrect trials. Error bars indicate the 95% confidence intervals for a repeated measures design (Loftus & Masson, 1994).
Figure 1.

![Graph showing proportion correct over sessions.](image)

![Graph showing number of correction trials over sessions.](image)
Figure 2

![Graphs showing the relationship between delay interval (s) and proportion correct, retention interval (s), and number of back lever presses for FR2 and FR5 conditions.]
Figure 3.

![Graph showing the proportion correct for Test1, Test2, and Test3. The graph indicates the proportion correct for 'Same' and 'Different' conditions across the tests.](image-url)
Figure 4

![Bar chart showing choice response time (s) for Correct and Incorrect responses across Test1, Test2, and Test3. The x-axis represents the tests, and the y-axis represents the choice response time. Error bars indicate the variability of the data.](image-url)