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Original Article

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Development of a Touch-Screen-Based Paradigm for Assessing Working Memory in the Mouse

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Assessing the working memory of the rodent by using a touch-screen system has several advantages (e.g., allowing highly accurate data collection and flexibility in memory task design). However, there is currently no available testing paradigm utilizing touch-screen systems that can assess working memory in the mouse. In this study, we developed a touch-screen testing paradigm in which mice were trained to choose a location that is matched to a sample location after a time delay. Consistent with previous studies, this study showed that mice could not only learn the rule in the delayed matched to position (DMTP), but also could retain a transitory memory of the sample position during delay. This indicates that a touch-screen system can provide a DMTP testing platform to assess working memory in the mouse.

Key words: touch screen, working memory, mouse cognition, delayed match to position

INTRODUCTION

Working memory is one of memory types, and it allows transitory information to be actively held for several seconds on line [1]. Several brain regions, such as medial prefrontal cortex, and hippocampus, are known to play critical roles in holding transitory memory [2,3]. Testing working memory in the mouse has typically used the delayed non-matched to position (DNMTP) paradigm [4]. This paradigm, however, has limited accuracy and flexibility of behavioral schedules. For example, during a working memory test in a T-maze, intervention by the experimenter is inevitable because the maze needs to be cleaned and the animal must be relocated to the start box after sampling. Therefore, there is a need to test working memory in operant-conditioning-based paradigm in which all experimental procedures will be scheduled automatically.

Touch screen testing is recently developed for assessing rodent's cognition. This touch screen paradigm has several advantages over traditional behavioral testing paradigms (e.g., Morris water maze [MWM], T-maze, and radial-arm maze). First, testing can be scheduled almost automatically, permitting more experimental sessions to be conducted with high accuracy and flexibility. Second, the method greatly reduces the need for experimenter intervention during memory test trials, because the experimental procedure is fully automated. Third, it can provide the animal with multiple choice locations on the screen, which enables the capability of assessing the animal's ability to discriminate between choice locations, such as pattern separation [5]. Fourth, various visual stimuli (e.g., from simple white rectangles to complex figures) can be presented on the screen. This enables testing of complex cognition, which was not able to test in the traditional behavioral tests [6]. These advantages would advance research on working memory, if touch-screen-based technology were to be applied. Unfortunately, a touch-screen system for the working

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memory test paradigm for mice is not available yet [7]. Therefore, in the present study, we developed a delayed matched to position behavioral assessment paradigm using a touch-screen testing system for evaluating working memory in mice.

MATERIALS AND METHODS

Animal

C57BL/6J mice were obtained from Orient Co. (Gyeonggi, Korea). Animals were housed in groups (four mice), maintained on a 12-h light/dark cycle, and food and water were provided ad libitum. All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Seoul National University. Male mice were used in behavioral experiments.

Touch-screen testing

We used Campden Instruments Bussey-Saksida touchscreen chamber (Campden Instruments Ltd, UK) for touch-screen testing. First, mice were deprived food to increase their motivation for reward. Food restriction started at age of 10 weeks, and their weights were maintained to about 80% of initial weight through all behavioral tasks. Before mice learned the rules of the cue-response paradigm and DMTP, they were trained to respond to visual stimuli on an LCD monitor to earn a reward. All experiments were conducted as described in the manufacturer's guide and previous studies [6-8]. Briefly, on day 1, mice were habituated to a touch-screen chamber for 10 min. On day 2, mice were given liquid sweetened milk as a reward. Liquid sweetened milk was made by diluting condensed milk (Seoulmilk, Korea) with the same volume of tap water. In this phase, the animal's nose poking to the reward magazine located behind the chamber resulted in delivery of reward. On day 3, in *initial touch* phase, both nose poking to reward magazine and response to visual stimulus on the LCD monitor delivered the reward (Fig. 1A). Next, on days 4 through 6, in the *must touch phase*, only when mice responded to the visual stimulus reward was delivered (Fig. 1B). After the *must touch phase*, mice were trained to avoid responding to a blank window; *incorrect punishment* (Fig. 1C). To train this avoidance, when the mice responded to a blank window, the room light was illuminated as a punishment signal. Sixty trials were conducted within 60 min until mice reached the criterion (70% correct response in consecutive 2 days).

After *incorrect punishment*, mice were trained to associate central cue and correct location choice for a reward. Two central visual cues have a different shape and color (Fig. 1). At the beginning of task, visual cue was presented in central window until mice touched visual cue. After central cue diminished, white squares were presented both in left and right windows. Guided by the central cues, mice could choose either the left or right location to receive a reward [6]. When the animal made an incorrect choice, the room light was illuminated, and additional correction trials were given until the animal made a correct choice. In cue-response training, 60 trials within 90 min were given for 5 sessions.

After cue-response training, same animals were trained to learn DMTP rule. Three touches to the left- or right-positioned sample/cue stimulus on the screen by the mouse resulted in presentation of a visual stimulus in a central window. When the animal responded to the central visual stimulus, visual test stimuli were presented both on the left and right side of the screen. The

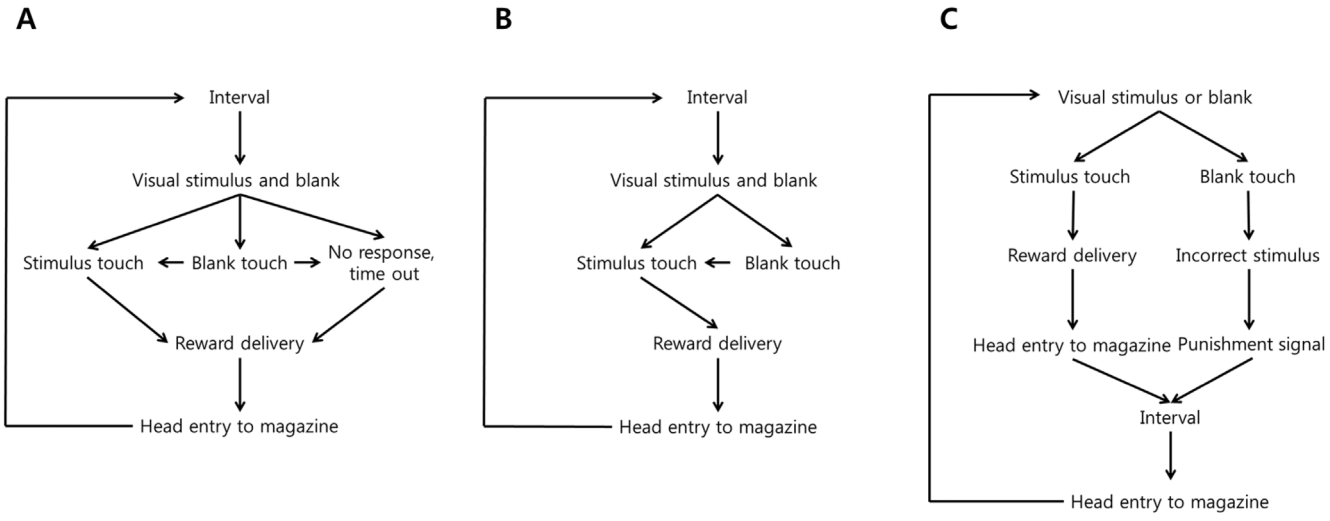

Fig. 1. Flowchart showing the behavioral schedule of operant conditioning. (A) Flowchart of initial touch, (B) Flowchart of must touch, (C) Flowchart of incorrect punishment.

mouse's correct matched-to-sample response delivered a reward. As in cue-response training, an incorrect choice resulted in room light illumination and additional correction trials. After successful DMTP rule learning, delay time between final response to the sample location cue stimulus and presentation of the central visual test stimulus was increased to either 3 s or 9 s.

RESULTS AND DISCUSSION

To test working memory, two types of transitory information can be used. First, in the "cue-delay-response" approach, the experimental animal makes a correct response guided by various cues presented at the start of the test trial. After the cue is diminished, the animal should hold a memory of the cue during the delay and up until the animal's choice moment is coming. To use this system, it is necessary to create a reference memory by which the cues can guide the animal to make a correct choice. Second, in the "delayed matched to position (DMTP)" or "non-matched to position (DNMTP)" paradigms, the animal should first learn the basic rule of the delayed *matched* or *non-matched* position to make a correct choice response. After the sampling location, a temporal delay is instituted before the animal is allowed to choose the matching (or non-matching) location.

To test working memory in a touch-screen system, we first tested whether mice can learn a reference memory in which they choose between locations on the left or right, following presentation of a visual cue presented in the center (Fig. 2). This task also has been used to test rodent's visuo-motor response [6]. On a given trial, to receive a reward, the mouse must touch a visual cue presented in the center location on the screen, and then choose either the left or right location of subsequent visual choice stimuli, in accordance with the visual cues (Fig. 2A) that have different colors and shapes. When choice moments were given 1 second after touching the center cue, however, mice performed poorly this task (Fig. 2B). Their failure to learn the reference rule indicates that using the cue-delay-response method in the touch-screen paradigm is not an appropriate way to test working memory in mice.

We next employed a DMTP paradigm in which working memory of mice can be assessed in a lever-pressing operant chamber [9], to determine whether mice can learn a DMTP rule (Fig. 3). To do sampling, the mouse was required to touch the visual stimulus presented in either the left or right position of the screen three times. One second later, the mouse was allowed to make a final sample touch of the visual cue presented in the center of the screen. After the mice made this final touch of the center position, they were then presented with two alternative visual stimuli that were positioned in both the left and right position. When mice chose a position that matched the cue's position, a sweet milk reward was delivered (Fig. 3A). When mice made an incorrect choice, correction trials were given until they made a correct choice. As was true in the lever-pressing operant chamber, mice successfully learned the matched to position rule (Fig. 3). After four training sessions, mice scored high in accuracy compared to their first session, and the number of correction trials also diminished (Fig. 3B), indicating that mice successfully learned the rule of DMTP (Fig. 3C).

After mice learned the matched-to-position rule, to test working memory, we increased delay time between final sample touch and central visual stimulus presentation (Fig. 4). When delay time was increased to 3 s, there was a slight, but significant, decrease in the

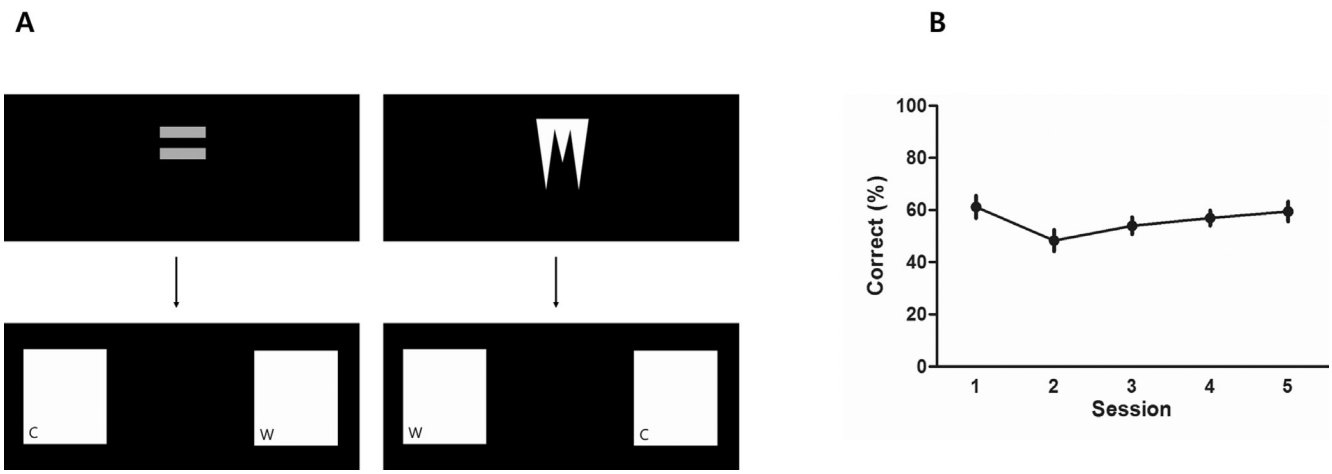

Fig. 2. Cue-response structure. (A) Schematic drawings of experimental design. Central cues used in this experiment were provided by manufacturer (Campden Instruments Ltd, UK). C and W indicate 'correct' and 'wrong', respectively. (B) When mice were guided by a cue to make the correct choice, they performed poorly in associating visual cue and correct choice location. Percentage of correct choice in the first session and last session were 61.3±4.3% and 59.5±3.4%, respectively. Error bars indicate SEM. Eight mice used in this experiment.

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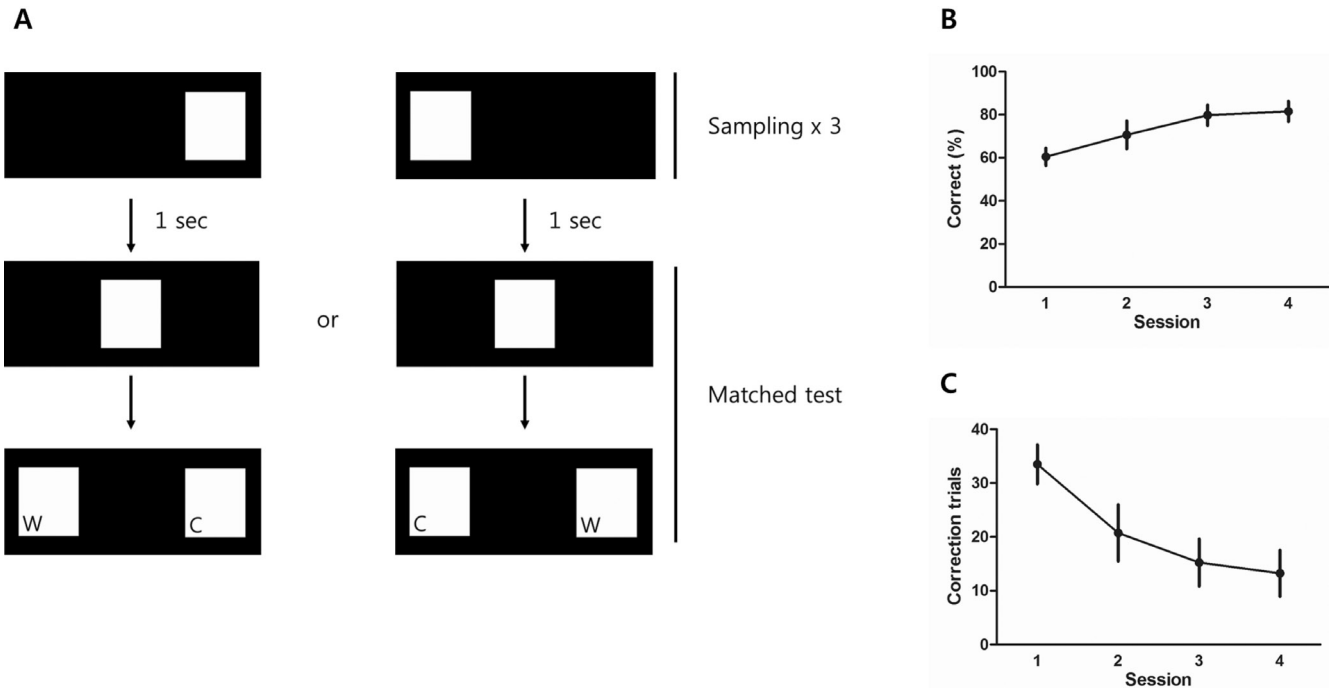

Fig. 3. Rule learning of DMTP. (A) Schematic drawings of experimental design. C and W indicate 'correct' and 'wrong', respectively. (B) Mice successfully made correct choices that match to sample location with session progression. Correct choice rate of the last session was significantly increased compared to that of the first session (paired t-test, $t(7)=6.524$, $p < 0.001$). (C) The number of correction trials required during DMTP training was significantly reduced (paired t-test, $t(7)=5.435$, $p < 0.05$). Error bars indicate SEM. Eight mice used in this experiment.

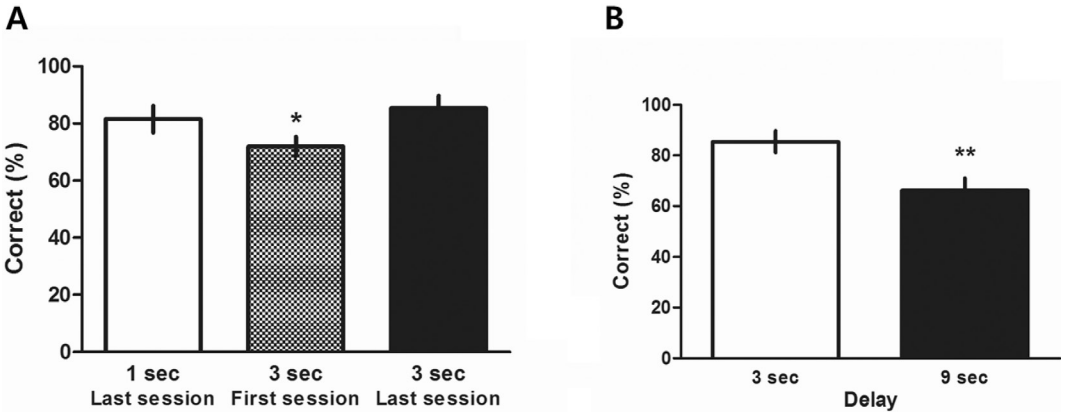

Fig. 4. Effect of prolonged delay in DMTP. (A) When delay time was increased from 1 s to 3 s, correct choice rate was slightly, but significantly, reduced (paired t-test, $t(7)=3.093$, $^{*}p < 0.05$). Percentage of correct choice in the last session of 1 s delay and the first session of 3 s delay were 81.5±4.7% and 71.9±3.4%, respectively. Repetition of DMTP task with 3 s delay increased correct choices comparable to a 1-s delay. (B) When delay was prolonged to 9 s, correct choice rate was significantly reduced (paired t-test, $t(7)=3.842$, $^{**}p < 0.01$). Percentage of correct choice in the last session of 3 s delay and 9 s delay were 85.4±4.3% and 66.2±4.9%, respectively Error bars indicate SEM. Eight mice were used in this experiment.

rate of correct choice to matched position (Fig. 4A), compared to the last session of the rule learning. When we further trained mice in a 3-s delay paradigm by twice, animals made correct choices comparable to those of the last session of rule learning (Fig. 4A). Finally, a 9-s delay resulted in a correct matched-to-position learning rate was substantially reduced compared to that in the last session of 3-s delay paradigm (Fig. 4B).

Prolonged delay time between final sample touch and central visual stimulus presentation reduced the rate of animal's correct matched-to-sample responses. For example, increasing delay time from 1 s to 3 s slightly reduced correct choice rate (Fig. 4). As more training sessions were given, however, mice made comparable

correct choices to those in the 1-s delay condition. This may indicate, in our experimental condition, that a 3-s delay is not long enough to test the working memory of mice. When delay time was increased to 9 s, mice showed substantially reduced correct choice rate, which indicates that their rate of correct choice of matched position is affected by delay time between sampling and choice moment.

As clearly mentioned in the previous report [7], trial-unique, delayed nonmatching-to-location (TUNL), the only known working memory testing paradigm using touch screen, failed to test working memory in mice. There are several differences between TUNL task and our current paradigm, which successfully assessed working memory of mice. Firstly, we employed DMTP paradigm, whereas TUNL task used DNMTP paradigm even though it is not clear how this difference affected cognitive performance. Secondly, we increased the number of sampling touch to 3 times as in lever pressing DMTP paradigm [9] whereas animals were allowed only one sampling touch in TUNL task. We suppose that this multiple sampling may increase retention of transitory memory during delay period. Thirdly, in our paradigm, mice were not required to do any specific task to initiate choice phase at the end of delay period, whereas in TUNL task, animals are required to poke reward magazine at the end of delay paradigm to initiate choice phase. This may also make our paradigm easier for mice to learn the rule of DMTP.

Our present approach to assess working memory in mice using touch-screen test could be used to characterize several features of working memory. First, how long mice can retain the memory of a sample position could be tested. Although not well investigated, 10 s is commonly used as the time limit in current T-maze-based experiments assessing working memory in mice [10]. Using a similar testing protocol in our present study, mice could make correct matched-to-sample choices with as long as a 9-s delay. Therefore, with our highly flexible and accurate system, the duration of transitory memory during DMTP tasks could be determined for mice. Second, the effect of pattern separation on working memory could be studied in mice [11-13]. The hippocampal CA1 region is required to make non-matched choices between highly separated choices but not between close choices in the radial-arm maze task. There is no study that assesses whether mice also have hippocampal CA1-dependent temporal separation memory. Using our present paradigm, it could be examined whether mice have temporal separation memory, and, if they do, which brain regions are involved in temporal separation memory.

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