Assessment of long-term working memory by a delayed nonmatch-to-place task using a T-maze

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Long-term working memory (LTWM) is a subdivision concept of working memory and indicates the enhancement of performance in a working memory task. LTWM has been shown in humans who have been engaged in a specific task requiring working memory over a long time. However, there is very little understanding of the exact mechanism of LTWM because of limitations of experimental methods in human studies. We have modified the standard T-maze task, which is used to test working memory in mice, to demonstrate LTWM in an animal model. We observed an enhancement of performance by repeated experience with the same working memory load in mice, which can be regarded as an LTWM. This effect seems to depend on the condition wherein a delay was given. This task may be a good experimental protocol to assess LTWM in animal studies.

Keywords: long-term working memory; T-maze; animal model

Introduction

Ericsson and Kintsch (1995) reported long-term working memory (LTWM) to be an expansion of working memory (WM) span by repeated training. They also suggested short-term working memory (STWM) as a term for what was previously considered 'working memory', which was the shortest-lived form of memory used in the processing of information for a specific task (Atkinson and Shiffrin 1968). Ericsson and Kintsch studied this LTWM with a specialized task for experts such as chess players, who can memorize arranged chess pieces, or waiters, who can memorize meal orders easily and over long periods (Ericsson and Kintsch 1995). After their initial study with this new aspect of WM, text production (Kellogg 2001), soccer games (Postal 2004), situation awareness (Sohn and Doane 2003), and other exercises that require mental skills have been studied with regard to LTWM.

However, we do not know whether this LTWM is an expanded STWM, is trained to be maintained for a longer time, or is a rapid transition from STWM to short-term memory (STM). The major reason for this poor understanding of LTWM is that research has been restricted to human studies. There are many useful, but invasive, techniques to investigate 'what happens in the brain' with higher resolution (at the cellular and molecular levels) that obviously cannot be used in human experiments. Thus, a task in which LTWM can be studied in an animal model would be advantageous in understanding the mechanism of LTWM.

The T-maze has been widely used to investigate specific aspects of spatial working memory (Weitzdoerfer et al. 2004; Takao et al. 2008), which is operationally defined as information that is only useful to a subject during the current experience with a task (Wenk 2001). A delayed nonmatch-to-place task has been widely used with rodents in various mazes, including the T-maze and radial arm maze. Here, we adjusted a delayed nonmatch-to-place task using a T-maze, to examine the possibility of investigating LTWM in an animal model. The task consists of two choices. After the first choice, the subject has to remember the first choice for a given delay to be rewarded at the second choice. We trained subjects with minimal delay to make them learn the 'rules', and then gave delays of several different durations between the two choices. The delay times are repeated once more after the first rotation. We found a phenomenon, which can be interpreted as an increase in working memory span by repetition of the same delay. This increase in success ratio by repetition can be regarded as acquisition of LTWM.

Materials and method

Materials

Animals

C57BL/6 male mice (8–11-weeks-old) were used. All animals were housed under a 12:12 light/dark cycle (lights off at 9 pm) with food available *ad libitum*.

All experiments were performed during the 'light' period. Water was restricted for 14–18 h before the task. After the task, water was provided *ad libitum* until the next water restriction. All care and use of animals were under approval of Institutional Animal Care and Use Committee in Seoul National University.

Reward

Petri dishes (diameter: 3 cm) with 100 µL of 10% sucrose were located at the end of each goal arm. To remove the possibility that the reward could be detected by sight, the Petri dishes were painted with a white marker pen. The sucrose was filled up at the start of each trial, so the subject could not get a reward if it chose the arm it had already visited.

Equipment

The T-maze was made of 5-mm-thick acryl. The maze design was modified from the design of Deacon and Rawlins (2006) and is presented in Figure 1A. The walls and bottom were gray in color. The bottom was slightly brighter than the walls so as to enable easy detection of black subjects. Guillotine doors were made of white acryl and were fixed through a hole and pin when the arm was opened. The maze was covered with four transparent acryl sheets – two above the goal arms, one above the start arm, and one above the start box to keep the mice inside. Each of them was linked to the wall by a hinge that can be opened easily.

Methods

Habituation

The mice were habituated to the experimenter for 4 days, 5 min per mouse. The subject mice were deprived of water for 14–18 h before each behavioral process per day, starting at the second day of habituation until the end of the whole behavior schedule. In the last 2 days, a Petri dish with a reward was also presented to mice in the transporter and home cage. For the following 2 days, mice were habituated to the T-maze by being exposed to the maze for 10 min, with each arm freely accessible and with rewards at the end of the two goal arms.
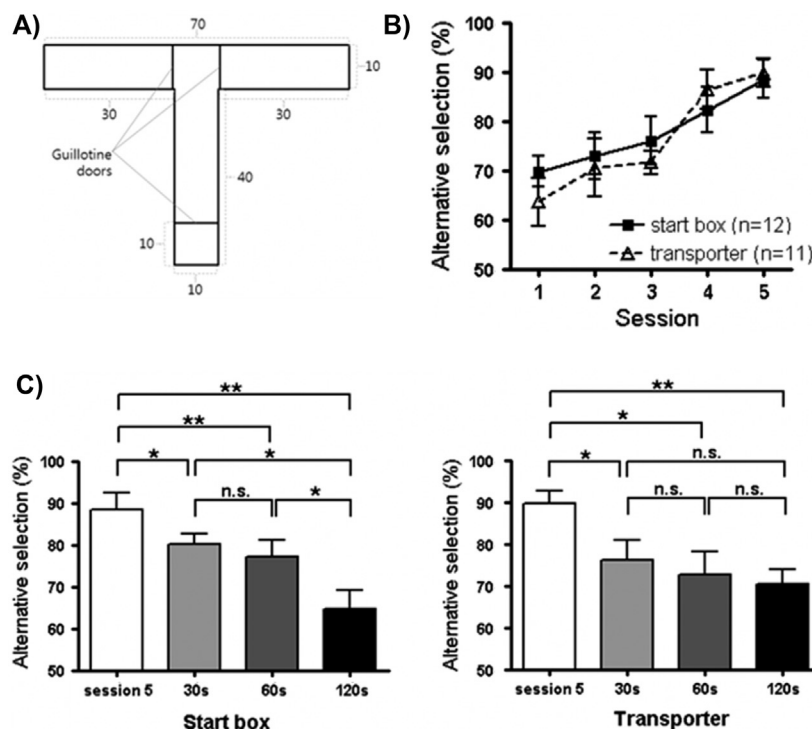

Figure 1.   (A) T-maze apparatus. Dimensions are in cm. The walls are 10 cm high. The horizontal arms are the goal arms and the vertical arm is the start arm. Guillotine doors can isolate each goal arm and the start box from the start arm. (B) Success ratio of alternative selection increased with training. We could see a ratio above 50% at session 1, and this ratio increased gradually over the training procedure. (C) In the delay trials, the subjects showed a lower level of alternation than that shown during session 5 of the training procedure in both the start box and transporter groups. The start box group had a significant decrease in the 120-s delay session, compared to the sessions with 30- and 60-s delays; the transporter group showed no significant difference between the delay sessions (*$P < 0.05$; **$P < 0.01$; n.s., not significant).

T-maze task

(i) Training. After habituation, the mice were trained for the T-maze task for five sessions. Each session consisted of eight trials – four trials per day. Intervals between trials lasted at least 30 min. Every trial was composed of two choices: forced choice and free choice. At the start of a trial, two rewards were located at the end of the goal arms, and a guillotine door for a sample arm was opened while the other doors were closed. After a subject was located in the start box, a guillotine door for the start box was opened to allow the subject to run to the sample arm and a door of the sample arm was closed immediately after its entering. Ten seconds were allowed for the mouse to consume the reward, and the mouse was removed from the arm to the start box or transporter as the last step of forced choice. The free choice was performed immediately after the forced choice. The free choice was the same as the forced choice except that the doors to the two goal arms were both opened at the beginning; the subject could choose either of the two goal arms. Once the subject selected and entered a goal arm, the door was closed for 20 s. Then, the subject was removed from the arm to the transporter and returned to its home cage.

(ii) Two turns of intervals. After 10 days (five sessions of training), the task was slightly modified; between the two choices, the mouse was kept in the start box or transporter for a certain delay period. The delay times were 30, 60, or 120 s, and four trials were performed for each delay time in random order during 3 days as a first turn. For the next 3 days, the same 3-day process was performed as a second turn. Eight trials of a given delay time were regarded as a session for that delay.

Data computation

When a mouse selected different arms during the forced and free choices, the trial was considered as a correct alternative selection. The percentage of alternative selections out of the eight trials was the representative percentage value for each session. One-way ANOVA with Newman-Keuls post-hoc test was performed among the training sessions and two-tailed paired *t*-tests were performed in the delay sessions.

To analyze the LTWM, we compared the four trials from the first turn with those from the second turn. For example, the 'first 30 seconds' consisted of four trials in the first turn. Paired two-way ANOVA was performed to analyze the delay length factor and repetition factor (the time factor for the first and second turns).

Results and discussion

Alternative selection increased in five sessions of training

The locations where the subjects received intervals were the start box of the maze or a transporter used to transport the mice for the experiment. The subjects were divided into two groups (the start box and transporter groups) on the basis of their interval location. The success ratio of alternative selection increased with training in both the groups (Figure 1B). We observed success ratios of 69.8% and 63.3% in the start box and transporter groups, respectively, after session 1. This higher-than-chance ratio is interpreted as spontaneous alternation, which is regarded as a characteristic because of the internal tendency of mice to explore new environments (Dember and Fowler 1958; Lalonde 2002). This spontaneous selection of the novel arm was strengthened by a reward given in that arm.

As the training proceeded, the success ratio increased gradually, with both groups showing similar levels. Since the working memory load was minimal between the forced and free choices, this improvement in performance is most likely based on the subject's understanding of the rule that there is reward in the arm that the subject has not been to, rather than an improvement in the subject's working memory ability. The subjects showed consistent ratios: >80% from session 4, with 88.5% and 89.8% at session 5 in the start box and transporter groups, respectively. A high success rate of 85–90% was reported in the T-maze task (Deacon et al. 2003; Deacon and Rawlins 2006). We consider that the mice had learned the rule sufficiently with minimal working memory load and were ready to be tested with a higher working memory load.

Alternative selection decreased in interval sessions

We performed the delayed T-maze task with three intervals. Delays of 30, 60, and 120 s were used randomly over the two turns and the results were compared with session 5 of training. As shown in Figure 1C, the subjects showed lower ratios of alternative selection in the delay sessions. This may be interpreted as an influence of higher WM load, as reported previously (Bolhuis et al. 1985; Masuda et al. 1992).

Although there was a significant difference compared to the last training session, which can be considered a 0-s interval, among trials with higher intervals a significant difference was seen only in the start box group, between 30 s and 120 s or between 60 s and 120 s. This decreased performance in longer intervals (i.e., higher WM load) indicates that this task is suitable for testing WM ability.

However, there was no difference among intervals in the transporter group. This may be because of complex factors such as attention or stress. The 'start box' protocol seems to be a better method to detect a gradual decrease in working memory performance as the memory load increased.

Success ratio increased in the second turn of intervals in the transporter group

Each session consisted of eight trials, conducted over 2 days. We analyzed the results between the first four trials and the last four trials to examine LTWM, indicating an effect of previous experience or training (Figure 2). The alternation ratios in the second turn were higher than that for the first turn for every interval, although there was not a statistically significant difference by the two-tailed paired *t*-test. However, a significant effect of repetition was observed in the transporter group by two-way ANOVA analysis ($P = 0.0196$). It seems that the complex factors related to the location where the intervals were given also played a role here, because the start box group did not show such a repetition effect. Nevertheless, the significant difference in the transporter group showed that the subjects enhanced their WM ability by the repeated experience. Different factors that can disturb the attention to maintaining working memory in the two conditions may have affected the improvement in performance.

Since WM is generally considered as the 'temporary storage and manipulation of the information necessary for cognitive tasks' (Baddeley 1992), improvement in that ability by repetition is different from the classic concept of WM. On the other hand, LTWM, according to Ericsson and Kintsch, explains this as the enhancement of WM span by exercise. The improved performance by repeated trials may be interpreted as acquisition of LTWM. Since there is no good behavior task to test LTWM in rodents so far, the repetition effect shown here may be a useful tool for studying the LTWM mechanisms by molecular and electrophysiological methods.

Delayed nonmatch-to-place task to study various aspects of LTWM

Although there was no significant difference between groups by an unpaired *t*-test, the start box group showed a stronger tendency to decrease as the interval duration increased. On the other hand, the transporter group showed a greater contribution of LTWM. Although the overall pattern seemed to be similar between the two groups, each group had a unique advantage to detect significant effects in independent aspects. The location where the subjects are kept for the interval should be chosen depending on the purpose of the experiment to maximize the resolution of the task.

Research on LTWM has been based on human studies. The T-maze task with interval repetition offers a method to access LTWM in the rodent model. A similar delayed nonmatch-to-place task using a radial arm maze may also work. Since a radial arm maze can provide a different aspect of increased memory load (i.e., an increased number of places to remember), it would be possible to investigate the different aspects of working memory capacity related to LTWM with a radial arm maze. By combining these tasks with other experimental techniques not applicable in humans, it may be possible to reveal the mechanism of LTWM. By lesion studies, it will be possible to examine regions involved in LTWM. With molecular analyses, it will be possible to examine the molecular changes related to LTWM. Finally, by combination with *in vivo* recording techniques, it should be possible to examine the neural activity patterns during LTWM, which would greatly improve our understanding of the mechanism of LTWM.
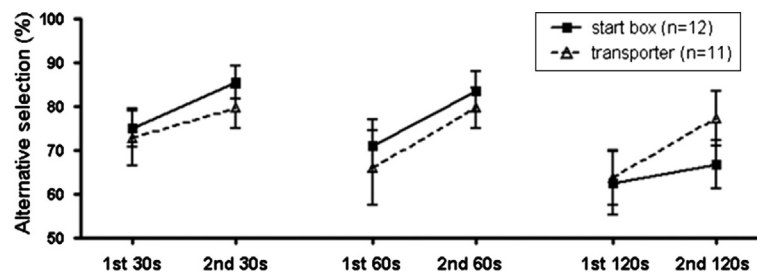

Figure 2.   In delayed selection, the average success ratios for alternative selection were higher at the second turn of each delay. We randomly used 30-, 60-, and 120-s delays during 3 days, and in the next 3 days the three delays were used again. Squares and triangles represent the groups that experienced the delay in the start box and transporter, respectively. There was a significant repetition effect for the transporter group ($P = 0.0196$).

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