Neuroscience Letters 497 (2011) 37–41

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Long-term object location memory in rats: Effects of sample phase and delay length in spontaneous place recognition test

Takaaki Ozawa, Kazuo Yamada, Yukio Ichitani*

Institute of Psychology and Behavioral Neuroscience, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

ARTICLE INFO

Article history:
Received 29 January 2011
Received in revised form 19 March 2011
Accepted 8 April 2011

Keywords:
Object location memory
Spontaneous place recognition
Spatial memory
Long-term memory
Rats

ABSTRACT

This study investigates the effects of sample phase and delay length on discrimination performance in the spontaneous place recognition (SPR) test in rats. Rats were allowed to explore an arena where two identical objects were presented for 5–20 min (sample phase). After a delay interval, rats were placed again in the same arena but one of the two objects was moved to a novel place (test phase). Results showed that when the sample phase was as long as 20 min, rats preferentially explored the moved object during the test phase even after a 6–24 h delay was interposed. Further sequential and cumulative analyses of the test phase revealed that the preference for the object in a novel place was evident in the first and 2nd min of the test phase in rats with a longer sample phase duration. Correlation analysis showed that locomotor activity and object exploration in the sample phase were not decisive factors in spatial memory performance. The present results demonstrate the importance of the sample phase exposure time and the test phase length.

Spatial memory is the ability of organisms to acquire cognitive representation of their location in space and thereby effectively navigate themselves to a specific goal in the environment. This type of memory has been assessed in many animal behavioral studies using various spatial learning and memory tasks; however, these tasks possess several disadvantages. Animals are required to learn task rules, which makes it difficult to differentiate their ability of spatial memory from their ability to acquire the rules of the task. Furthermore, utilization of a food reward or aversive stimuli as a reinforcer can affect the animal's physical, motivational and emotional states, which make it difficult to determine the specificity of an experimental manipulation.

Spontaneous "place" recognition (SPR) test, also called spontaneous object location recognition or object location test, has been developed for the assessment of spatial memory. This test takes advantage of the rats' innate tendency to spontaneously explore objects in a novel place [4]. In the standard procedure of this task, a rat is allowed to freely explore an open-field arena with two identical objects for several minutes (sample phase). After a delay period, the rat is returned to the arena with the same two objects, but one object is positioned in a novel location while the other is in its original location. During this test phase, if the rat spends more time exploring the object in the novel location, then this rat is considered to have spatial memory of the original position of the two objects during the sample phase.

Although the SPR test is widely used, there are only a few studies focused on the SPR test with a delay interval longer than 24 h [5,7,8]. In the present study, we investigated whether rats exposed to two objects during sample phase for different durations (5–20 min) could show clear evidence of SPR memory after a 6 or 24 h delay interval. If the preference for an object in a novel location reflects the existence of spatial memory of object location, then rats that experienced a longer duration of object exposure during the sample phase should show significant novelty preference in the test phase even after a longer delay, since longer exposure is thought to produce stronger encoding of spatial information of objects. Secondly, most studies using the SPR test analyze the discrimination score for a whole period in the test phase [4,9,10]. Here, we investigated how the discrimination between objects varied within test phase by performing sequential and cumulative analyses of object discrimination during the 5 min test phase. Lastly, we examined the relationship between exploration of objects in the sample phase and discrimination performance in the test phase.

Twenty-six male Wistar–Imamichi rats (8 weeks old) were used as subjects. Mean body weight at the start of experiments was 298 g. Rats were housed in individual cages with a 12:12 h light–dark cycle and food and water were provided *ad libitum* throughout the experiments. Animal experiments were approved by University of Tsukuba Committee on the Animal Research. All efforts were made to minimize the number of animals used and their suffering.

* Corresponding author at: Institute of Psychology, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan. Tel.: +81 29 853 6094; fax: +81 29 853 6094.
E-mail address: y-ichit@human.tsukuba.ac.jp (Y. Ichitani).

0304-3940/$ – see front matter
doi:10.1016/j.neulet.2011.04.022

An open-field arena (90 cm × 90 cm × 45 cm) made of polyvinyl chloride was used and the walls of the arena were colored in black, while the floor was gray. On one of the walls, a white–black vertically striped pattern was placed as an absolute spatial cue. The objects employed were black triangular cast metals, white cylinders of cast metal, cans of juice, and brown china bowls. All objects were heavy enough or fixed to a heavy metal plate so that rats could not move them during testing. A video camera was suspended above the arena, and images were projected to a monitor to allow the experimenter to observe the animal's behavior.

Handling and habituation to the apparatus preceded the SPR test. Rats received 5 min of handling for 3 days and were habituated to the apparatus by freely exploring the arena for 10 min for 4 days. One trial of the SPR test consisted of a "sample phase" and a "test phase", and these phases were separated by a delay interval. In the sample phase, two identical objects were placed diagonally in the arena (the center of each object was 22.5 cm from adjacent two walls), and a rat was released in the center of the arena and left to freely explore the open-field. After a pre-defined sample duration, the rat was removed and then returned to its home cage where it remained for a pre-defined delay interval. After the delay, the rat was returned to the arena (test phase). In this phase, one object was placed in the same original position as in the sample phase (object F), while the other was moved to a different position (object N), which was 30 cm away from object F and 22.5 cm from a sidewall (two locations were possible).

In this study, three sample phase durations (5, 10 and 20 min) and two delay lengths (6 and 24 h) were used. Rats were assigned to either 6 h-delay group ($n = 16$) or 24 h-delay group ($n = 10$) and within each delay group, rats were tested three times with different sample phase durations in a random order. The duration of test phase was always 5 min. There was 48 h minimum interval between trials. Different pairs of objects were randomly used in each trial. After each phase, the floor of the arena and the objects were cleaned with 70% ethanol.

In each phase, we measured the time rats spent exploring the objects. Exploration was defined as the rat directing its nose toward each object within a distance of 2 cm. As a measure of discrimination, "discrimination index (DI)" was calculated by dividing the difference in exploration time between the two objects (object N–object F) by the total amount of exploration for both objects (object N + object F). DI was then multiplied by 100 to express as a percentage. Additionally, locomotor activity was analyzed with Image OF (O'Hara, Tokyo), a software modified from the NIH image program (developed at the U.S. National Institutes of Health).

For statistical analysis, the time spent exploring each object during the sample phase was analyzed with a three-way analysis of variance (ANOVA) (delay × sample phase duration × object) with repeated measures, followed by post hoc comparisons using a Fisher's LSD test ($p < 0.05$). Object exploration time during the 5 min test phase was first divided into separate 1 min bins. Then, the DI of each 1 min bin was calculated and compared to chance level (0%) using a one-sample t-test. Additionally, the effect of sample phase duration on discrimination was analyzed cumulatively. DI data obtained from each 1 min bin during the test phase were cumulatively calculated for the first 1, 2, 3, 4, or the total 5 min duration of test phase and analyzed with a two-way ANOVA (delay × sample phase duration) with repeated measures, followed by post hoc comparisons using a Fisher's LSD test ($p < 0.05$). DI data were also compared to chance level (0%) using a one-sample t-test. Furthermore, the total amount of time spent exploring the objects in the test phase in each condition was compared using a two-way ANOVA (delay × sample phase duration) with repeated measures, followed by post hoc comparisons using a Fisher's LSD test ($p < 0.05$). In previous studies, the relationship between object exploration time during the sample phase and the magnitude of recognition memory has been examined using the "difference score (DS)" as a measurement of recognition memory [1,6]. DS is calculated as the difference in exploration time between the two objects in the test phase (object N–object F). For consistency with previous studies, we also used DS in the correlation analysis. The relationship between total amount of exploration time or total moving distance in the sample phase and either DI or DS data obtained from the first 1, 2, 3, 4 or the total 5 min test phase was examined using the Pearson's product-moment correlation coefficients.

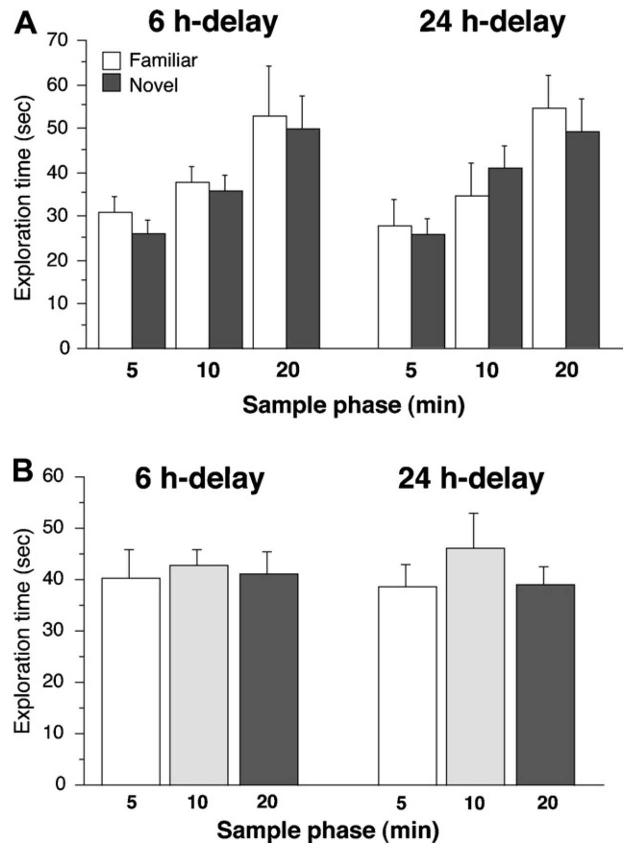

Fig. 1. (A) Exploration time in the sample phase. "Familiar" and "novel" mean the object that will be in the same place and the object that will be in a novel place in the test phase, respectively. (B) Total exploration time in the test phase (5 min). Horizontal axis indicates the length (min) of sample phase. Mean ± SEM are shown.

Exploration time of each object during the sample phase is shown in Fig. 1A. Three-way ANOVA showed a significant main effect of sample phase length ($F(2, 48) = 11.56$, $p < 0.001$). Subsequent post hoc tests revealed that exploration time of objects was greater in the 20 min-sample condition than in 5 and 10 min conditions ($p < 0.05$) and greater in the 10 min-sample condition than in the 5 min condition ($p < 0.05$). There were no significant main effects of delay length or of objects to be later placed in the familiar or novel location in the test phase. Thus, rats explored both objects equally, regardless of sample phase length and subsequent delay length. It was also shown that even if the sample phase was extended, rats continued to explore the object for the entire 20 min of testing. However, since the exploration time in the 20 min-sample condition was less than fourfold of the exploration time in the 5 min condition, exploration of the objects appeared to have decreased over time. For the test phase, the total amount of time spent exploring objects is shown in Fig. 1B. Two-way ANOVA revealed no significant effects of sample phase duration or delay length.

Fig. 2 shows the time course of DI in the test phase under each combination of sample phase length and delay condition.