Research report

The diurnal variation of performance of the novel location recognition task in male rats

Yoshiyuki Takahashi [a], Kosuke Sawa [b], Takashi Okada [a],*

[a] Department of Psychology, Faculty of Human Sciences, Sophia University, 7-1 Kioi-cho, Chiyoda-ku, Tokyo 102-8554, Japan
[b] Department of Psychology, School of Human Sciences, Senshu University, 2-1-1 Higashimita, Tama-ku, Kawasaki, Kanagawa 214-8580, Japan

HIGHLIGHTS

- We examined the circadian rhythm of performance in the novel location recognition (NLR) task using rats.
- The performance was better during the dark period than during the light period.
- Artificial change of melatonin level modulated the diurnal variation of performance.

ARTICLE INFO

ABSTRACT

Circadian changes of performance have been reported in various kinds of learning task. However, the diurnal variation of performance in hippocampus-dependent learning tasks remains unclear. In the present study, rats were subjected to the novel location recognition (NLR) task as well as the novel object recognition (NOR) task to examine whether the circadian pattern of hippocampus-dependent task performance was similar to that in tasks in which brain regions other than the hippocampus contribute. The performance in the NOR task was relatively constant irrespective of the time of day, while the performance in the NLR task was higher at night than during the daytime. When the pineal hormone melatonin was injected into rats before the training phase in order to examine its effects on the pattern of circadian changes of NLR performance, rats showed improvement of performance in the daytime, but impairment at night. These results suggest that the pattern of circadian variation of memory performance depends on the type of task, and that the effects of exogenous melatonin on learning performance vary with the time of day.

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1. Introduction

Circadian rhythms are widely observed in animal physiology and behavior, and memory performance reportedly varies with diurnal time [1]. However, the identified patterns of diurnal variation of memory performance differ among previous studies. One study showed that memory performance was better during the light period than the dark period in fear conditioning in mice [2]. Contrarily, performance of the novel object recognition (NOR) task was better in the dark period than the light period in hamster [3] and task performance using the Morris water maze showed no difference between light and dark periods in rats [4]. One possible reason for this discrepancy is the difference of brain regions that play an important role in each task. For example, it has been pointed out that the amygdala plays an important role in Pavlovian

fear conditioning [5], and that hippocampal and/or perirhinal regions are important for the NOR task [6–8].

In this study, we focused on the diurnal variation of performance in hippocampus-dependent learning tasks. Since hippocampal long-term potentiation (LTP) is regarded as one of the physiological bases of memory and learning [9–11] and since the magnitude of hippocampal LTP shows diurnal variation [1], the diurnal variation of hippocampal LTP magnitude may be reflected in the diurnal variation of performance in hippocampus-dependent tasks. Although it was proposed that the NOR task is hippocampus-dependent [6], a recent study has indicated that the novel location recognition (NLR) task, rather than the NOR task, is hippocampus-dependent [8]. Since the diurnal variation of performance in the NLR task has not been reported, we examined animal performance in not only the NOR task but also the NLR task in this study.

Another purpose of this study was to examine whether melatonin affects diurnal variation of NLR task performance. Melatonin, a hormone mainly secreted by the pineal body, shows a circadian pattern of secretion: there is more secretion at night than during the day in both nocturnal and diurnal animals [12]. Since melatonin

* Corresponding author. Tel.: +81 3 3238 3981; fax: +81 3 3238 3811.
E-mail addresses: okadat@sophia.ac.jp, okada.takashi@nifty.ne.jp (T. Okada).

was shown to decrease the magnitude of LTP in the hippocampal CA1 region [13,14], it is one of the endogenous candidates of regulators of hippocampal synaptic plasticity and animal learning performance according to the time of day. As for the effects of melatonin on learning performance, melatonin treatment before the test phase in the light period enhanced the NOR task performance in rat [15]. However, the effects of melatonin on memory performance in the NLR task remain unclear. We examined the effects of exogenous melatonin on memory performance in the NLR task both in the daytime and at night.

2. Material and methods

2.1. Subjects

Six-week-old male Wistar rats were purchased from CLEA Japan (Tokyo, Japan). The rats were housed under a daily light/dark cycle, which consisted of 12 h of light conditions (started with lights on at 7:00 a.m.; defined as ZT0) and 12 h of dark conditions (started with lights off at 7:00 p.m.; defined as ZT12). Rats were handled for about 5 min at ZT10-12 before experiments for 3–7 consecutive days. Then, experiments were begun more than 20 h after the end of final handling. The rats could drink water freely, and the diet was restricted in order to ensure sufficient exploration time throughout the experiments [16]; about 20 g/day diet was fed at ZT10-12 before the experiments for 2–3 days. To check the effects of handling and feeding time on NLR task performance, handling and feeding were conducted at ZT22-24 and experiments at ZT20 in a certain group (Experiment 2). In Experiment 3, handling and feeding were conducted at ZT10-12 and ZT22-24 in ZT8 and ZT20 groups, respectively. All animal procedures were approved by the Institutional Animal Care and Use Committee of Sophia University.

2.2. Apparatus

A plastic black box (50 cm in length, 44 cm in width, and 50 cm in height) was used in the experiments. Objects that rats should explore were made by combining Lego® blocks, to ensure consistency in the material (plastic), color (white), and size (6.4 cm in length, 6.4 cm in width, and 8 cm in height) of each object (Fig. 1). The apparatus was enclosed with a black curtain, so rats could not use any visual cues outside of it.

2.3. Procedure

2.3.1. Experiment 1: NOR task

Taking the diurnal variation of the endogenous melatonin secretion pattern [17] into consideration, experiments were conducted at one of four ZT timings (ZT2, ZT8, ZT14, or ZT20). The NOR task consisted of an adaptation phase, a training phase, and two test
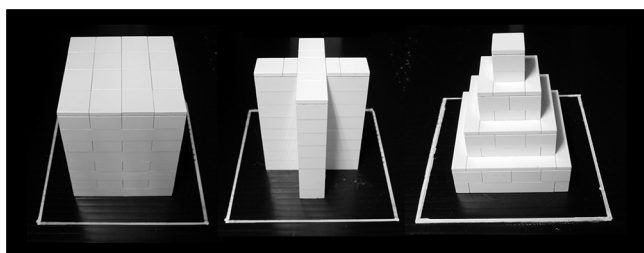

Fig. 1. Objects used in the NOR and NLR tasks. Three shapes of object (square pillar, pyramid, and cross pillar) were made from Lego® blocks, to ensure the same texture (plastic), color (white), and size (length 6.4 cm, width 6.4 cm, height 8 cm). Three objects were used in the NOR task, and square pillar and cross pillar objects were used in the NLR task.

phases. In the adaptation phase, the rats were allowed to explore the inside of the apparatus and a sample object (made up of white Lego® blocks, 12.8 cm in length, 1.6 cm in width, 2.2 cm in height; placed in the center of the box) for 10 min to ensure acclimation to the apparatus and Lego® blocks. The training phase was begun at the timing of 24 h after the onset of the adaptation phase. In the training phase, two identical objects were placed in adjacent corners of the apparatus, 10 cm away from the two adjacent walls, and rats could explore the inside of the apparatus for 10 min. Then, the short-term memory (STM) test phase was conducted at the timing of 1 h after the onset of the training phase. In this phase, two objects were placed in the same location as in the training phase. One of these two objects had an identical shape to that used during the training phase ("familiar object"), while the other had a different shape ("novel object"). The rats were allowed to explore the inside of the apparatus for 5 min. In addition, at 24 h after the onset of the training phase, a long-term memory (LTM) test phase was conducted. In this phase, two objects were placed in the same location in the apparatus as during the training phase. One of these objects was a familiar object used in the training phase, while the novel object (shown in the STM phase for the first time) was replaced with an object with another shape. The rats were allowed to explore the inside of the apparatus for 5 min. They were then returned to their home cages just after the offset of each phase, and the cages were returned to the breeding rack. We should note that, in the present procedure, not only the training phase but also the STM test phase served as the acquisition phase for the LTM test.

The apparatus and objects were cleaned using paper with about 15% ethanol solution after each phase. The shape of the objects and the location of the replaced object were counterbalanced.

2.3.2. Experiment 2: NLR task

To examine the effects of the time of day on learning performance in the NLR task, experiments were conducted at one of four ZT timings (ZT2, ZT8, ZT14, and ZT20), as in Experiment 1. The NLR task also consisted of an adaptation phase, a training phase, and two test phases. The procedures of the NLR task were identical to those of the NOR task, except for the location. In the training phase, the objects with different shapes were placed in diagonally opposite corners of the apparatus (10 cm away from the walls). Then, throughout the test phases, one of two different objects was placed in the same location used in the training phase ("familiar location"), while another object was placed in different locations ("novel location") in each of the STM and LTM tests. In the NLR task, a sheet of cat repellent mat (length of plastic spikes: 3 cm, height and width of the mat: 13 cm and 50 cm, respectively) was fitted on one side of the wall of the apparatus as a spatial orientation cue for rats to use in the apparatus.

2.3.3. Experiment 3: Melatonin treatment

To examine the effect of melatonin on performance in the NLR, either saline or saline with melatonin solution was injected into rats (i.p.). The timing of the injection was 30 min before the onset of the training phase or just after the end of the training phase. The procedures were similar to those in Experiment 2, except for the injection of solution.

The timing of handling and feeding under each ZT condition is shown in Fig. 2.

2.4. Drugs

Melatonin was purchased from SIGMA (St. Louis, MO, USA). It was dissolved in saline (0.9% NaCl) at 10 mg/mL and cryopreserved. Melatonin and saline were i.p. injected at 10 mL/kg [15].