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Research report

Brief hind paw stimulation is sufficient to induce delayed somatosensory discrimination learning in C57BL/6 mice

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HIGHLIGHTS

- A new somatosensory working memory task was developed for head-fixed mice.
- Stimulation to left hind paw was sufficient to induce discrimination learning.
- Mice were capable of performing discrimination reversal learning.
- The somatosensory working memory span in mice was estimated to be at least 10 s.

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ABSTRACT

Somatosensory learning and memory studies in rodents have primarily focused on the role of whiskers and the barrel structure of the sensory cortex, characteristics unique to rodents. In contrast, whether associative learning can occur in animals (and humans) via foot stimulation remains unclear. The sensory cortex corresponding to the plantar foot surface is localized in the centroparietal area, providing relatively easy access for studying somatosensory learning and memory. To assess the contribution of sole stimulation to somatosensory learning and memory, we developed a novel operant-lever-pressing task. In Experiment 1, head-fixed mice were trained to press a lever to receive a water reward upon presentation of an associated stimulus (S+). Following training, they were administered a reversal-learning protocol, in which "S+" and "S−" (a stimulus not associated with reward) were switched. Mice were then submitted to training with a progressively extended delay period between stimulation and lever presentation. In Experiment 2, the delayed discrimination training was replicated with longer delay periods and restricted training days, to further explore the results of Experiment 1. When the stimuli were presented to a single left hind paw, we found that male C57BL/6J mice were capable of learning to discriminate between different foot stimuli (electrical or mechanical), and of retaining this information for 10 s. This novel task has potential applications for electrophysiological and optogenetic studies to clarify the neural circuits underlying somatosensory learning and behavior.

1. Introduction

The somatosensory system is one of the most crucial sensory systems allowing animals to recognize the environment. In contrast to other sensory modalities, such as vision, audition, and olfaction, somatic sensation provides proximal information to detect both the physical nature and spatiotemporal pattern of objects [1,2]. Furthermore, tactile stimulation is closely associated with nursing, affiliative, and social behavior in mammals [3,4]. Thus, a large number of studies have been conducted to elucidate the neural and molecular mechanisms underlying somatic sensation in wide range of mammals, including humans [e.g.,5–8].

Rodent studies have primarily focused on the whiskers due to the distinctive structure of their corresponding primary sensory (barrel) cortex, and to the functional properties (e.g., fine movement and high sensitivity) of the whiskers. A number of reports regarding somatosensory learning and memory via whiskers have been published [9–12]. However, in spite of the intensive investigation of place conditioning using "tactile cues" [e.g.,[13], the contribution of foot sensation to learning as a cue stimulus remains unclear. In home cages, both the paws and whiskers are in constant contact with environmental stimuli, including the floor, walls,

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bedding, and food. Thus, somatosensory stimuli from the paws may play significant roles in the behavior of these animals, with respect to both basic reflexes and higher cognitive functioning [14]. Furthermore, foot sensation is common to rodents and humans, while the whisker-barrel cortex structure is unique to rodents. Therefore, studying the role(s) of foot sensation in learning and memory in mice may provide useful clues to human somatosensory learning and memory as well.

Working memory, a form of non-permanent storage [15], is one of the most frequently measured parameters in the analysis of mammalian neural circuits and higher brain function [e.g.,16–18]. Working memory is assumed to be based on the temporal activation (and/or inactivation) of neurons [reviewed in Ref. 19]. A number of behavioral tasks have been established to study working memory, including the eight-arm radial maze and the delayed matching-to-sample task [reviewed in Ref. 20]. These behavioral tasks are powerful tools that assess the cognitive functions of animals, including working memory, but present disadvantages in the need for an excessively large number of training sessions and incompatibility with experimental methods requiring head fixation or other movement restriction (e.g., neuroimaging). In electrophysiological studies of rats and mice, the "*Go*/*No-Go* task" has often been used to assess learning, memory, and other neural functions [21–23]. In this task, two different stimuli are typically used, and animals are required to perform a predetermined response (for example, pressing a lever or licking) when the *Go* stimulus is presented during a specified time window, or to inhibit this response when the *No-Go* stimulus is presented. Many visual, auditory, and olfactory cues have been used as *Go* or *No-Go* stimuli [24–27]. O'Conner and colleagues showed that mice could successfully learn *Go*/*No-Go* tactile object localization using a brief tactile stimulus to the whiskers in head-fixed mice [11]. Although this study involved spatial, rather than tactile, discrimination, their results indicate that the *Go*/*No-Go* task is a valid technique to investigate the learning ability of mice in a head-fixed paradigm.

In the present study, to develop an experimental system compatible with head-fixed protocols and translatable to human and primate analyses, we validated a novel protocol in which mice were trained to perform a *Go*/*No-Go* task using foot stimuli as signals.

2. Materials and methods

Animals

Twenty male C57BL/6 J JmsSlc mice (Japan SLC, Shizuoka, Japan) were used ($n = 10$ each for Experiments 1 and 2, respectively). Mice were purchased at 7 weeks of age. After 1 week of acclimation to the breeding environment and handling procedures, they were moved to individual housing, and surgeries were performed as per Section 2.2. Following 1 week of recovery, mice were water-restricted, with drinking allowed at a level to maintain 85% of their normal body weight.

The breeding and experiment room was continuously air-conditioned (with a temperature of $23 \pm 2\,^{\circ}C$ and a humidity of $55 \pm 10\%$). Animals were housed in a clear plastic cage ($27.5 \times 17 \times 12.5$ [height: H] cm) with paper bedding (TEK-FRESH, Harlan Laboratories Japan, Tokyo, Japan), and a light–dark cycle of 12:12 h (with lights on at 07:00). Food (CRF-1, Oriental Yeast Japan, Tokyo, Japan) was freely available except during training. Supplemental water (with an amount adjusted for each mouse every day) was provided to mice at least 1 h after the termination of the daily training to maintain body weight.

All animal experiments in the present study were conducted in strict accordance with the guidelines of The Institute of Physical and Chemical Research (RIKEN), and were approved by the Animal Investigation Committee of the institute.

2.1. Surgery

Mice were anesthetized with isoflurane (Wako, Osaka, Japan) using an inhalation anesthesia apparatus for small animals (SN-487-0T, Shinano, Tokyo, Japan). After stable, sufficient anesthesia was achieved, each mouse was placed on a fixation device (SG-3N, NARISHIGE, Tokyo, Japan), and a metal plate (made of titanium; $1.5 \times 3.8 \times 0.1$ cm; 2.0 g; with a 1 cm diameter hole at the center of the plate) was attached to the top of the skull using dental cement (Dental Adhesive Resin Cement, Shiga, Japan). The body temperature of the animals was maintained at 37 °C throughout the surgery using a keep-warm bag ("YUTAPON", Hakugen, Tokyo, Japan). Mice recovered in their home cages for 1 week with freely available food and water.

2.2. Experimental settings

Experimental devices used in the present study were custom-made by Daiwa Riken (Saitama, Japan). A head-fixing block for the mice was made of aluminum ($25 \times 20 \times 15$ [H] cm), with some attachments assembled in our laboratory including a retractable lever, a retractable drinking spout, two nickel electrodes to supply electric foot stimuli, and a solenoid to provide the mechanical foot stimulus (Fig. 1A). The retractable lever had a movement range of approximately 1 cm forward from the home position. Similarly, the retractable drinking spout had an upward movement range of approximately 1.5 cm, and provided a drop of filtered water by a peristaltic pump (WP1000, WELCO, Tokyo, Japan). Two types of discriminable foot stimuli were delivered on the top of the left foot stand. An electric foot stimulus was administered by a stimulus generator made in our laboratory (70 V AC, 0.26 mA; 5 m s × 2, with an inter-stimulus interval of 40 m s; Fig. 1B). A mechanical foot stimulus was supplied by a solenoid (24 V DC; driven at 9 V DC), which applied pressure to the plantar surface of the foot with a slim iron core (stimulus duration, 50 m s; Fig. 1C). All experiments were conducted in a light-controlled, sound-proof chamber (model ENV-0185, Med Associates, St. Albans, VT, USA; 150 lux at the center of the floor; the noise from electric fan was about 50 dB in the chamber), to reduce interference or distraction caused by outside noises. Inputs of animals' responses and outputs of stimuli were controlled by a microcomputer board (Arduino UNO; SWITCHSCIENCE, Tokyo, Japan), and other experimental controls and data collection were operated by a Windows PC. The fixing block and its attachments were cleaned using 70% ethanol after the training protocol of each mouse was completed.

2.3. Training procedure

Prior to discrimination training, each animal received a series of habituation training sessions to the experimental conditions and lever-pressing protocols. Mice were fixed to the experimental apparatus (the head-plate was bolted on the fixing block, the hind paws were held with adhesive tape on the foot stands, and the body was supported by a sponge cushion; see Fig. 1A), and allowed to press the lever to receive a small amount of water (20 μl). The training schedule was a continuous reinforcement schedule (CRF), in which mice received water as a reward every time they pressed the lever. Mice were required to engage in more than 50 lever-press responses during the habituation session (with a cutoff time of 30 min). Habituation training continued until mice could press the lever more than 50 times within 30 min for 3 consecutive days (which was defined as the habituation criteria). When a mouse reached the habituation criteria, discrimination training was