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Deterioration of Spatial Learning Performances in Lipopolysaccharide-Treated Mice

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ABSTRACT—It is well demonstrated that acute or chronic stress leads to reduction of learning ability. Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria, induces profound physiological and behavioral changes, including fever, decrease in food motivation, and decrease in social behavior. These changes might be interpreted as an acute stress reaction to the LPS. In the present study, therefore, we investigated the effects of LPS (400 – 800 μg/kg, i.p.) on spatial learning performances using C57BL/6J male mice. In the Morris water-maze task, spatial learning performances were examined in six trials of training for two consecutive days. LPS-treated mice took a longer time to reach the hidden platform than control mice ($F(1,60) = 4.80801$, $P<0.05$ at 600 μg/kg). In addition, injection of LPS decreased the percent of correct choices in the Y-maze test ($P<0.05$ at 800 μg/kg). LPS, however, did not alter the body weight, grip tone, motor activity or swimming speed. Taken together, these results indicate that LPS treatment specifically impaired spatial learning performances.

Keywords: Spatial learning, Stress, Lipopolysaccharide

Lipopolysaccharide (LPS), a product of the cell wall of gram-negative bacteria, is the active fragment of endotoxin. LPS has been found to induce infection-like sickness symptoms in experimental animals as well as humans. LPS administration results in physiological and behavioral disturbances; including fever, anorexia, body weight loss, induction of slow-wave sleep, suppression of locomotor and exploratory activity, decrease in food motivation and reduction in social and sexual behavior (1 – 8).

Proinflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α, are released from the activated immune cells in response to LPS. Peripheral administration of LPS also increases the brain level of IL-1β, IL-6 and TNF-α (9, 10). Additionally, peripheral stimulation with LPS has been reported to increase serotonergic and noradrenergic neurotransmitters (11). Stressors produce many of the same neuronal and endocrine responses as those that follow LPS. It is now well established that stress impairs learning performances. A large number of studies have been made on the effects of LPS (1 – 8); however, little is known about the effects on learning performances.

Therefore, in this paper, we intended to investigate the effects of LPS on learning performances in mice. We used the Morris-type water maze and the spontaneous alternation Y-maze tests, both of which are classified as a spatial learning task. Spatial learning requires integrative control functions of hippocampus (12). Peripheral administration of LPS activates the immune system and neurotransmission in the hippocampus as well as the other brain regions (13 – 17). To further examine the effects of LPS on learning performances, we tested the step-through type passive avoidance task, an index of learning and memory of distasteful experience (18).

MATERIALS AND METHODS

Animals and drugs

Male C57BL/6J mice (Japan SLC, Hamamatsu) of 6 – 8-weeks old were used in all experiments. Mice were housed individually under conditions of controlled temperature and humidity (22 ± 1°C, 55 ± 5%) with ad libitum access to food and water. Lipopolysaccharide (0127:B8, lot. n. 63H-4010; Sigma Chemical Co., St. Louis, MO, USA) diluted in the saline was intraperitoneally (i.p.) injected.

Spatial learning tests

Morris-type water maze test: The water maze pool was

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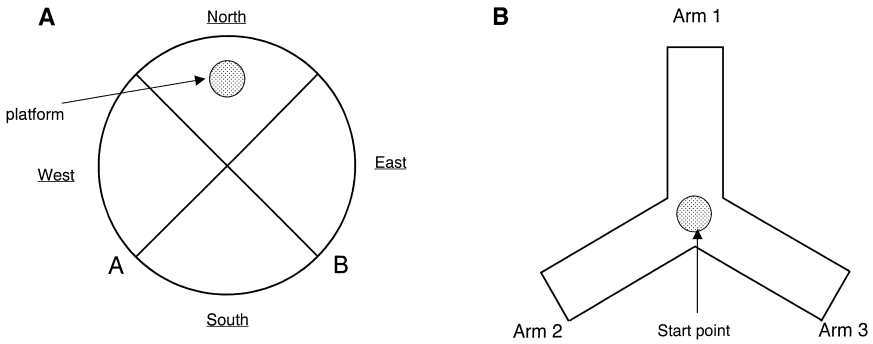

Fig. 1. Schematic drawings of water maze (A) and Y-maze (B) test apparatus.

a circular blue tank of 68 cm in diameter and 32 cm in depth. The pool was filled with tap water (19 – 20°C) so that water depth was 27 cm, and placed in a large testing room, which was furnished with various cues for spatial orientation. These materials were not moved throughout the period of experiment. Positions of the platform and start points (A and B) are described in Fig. 1A. The movement of each mouse in the pool was recorded with a video camera, and analyzed with a computerized tracking system. Prior to the experiment, each mouse was placed in the pool and allowed to swim freely for 60 s without a platform. On day 0, a circular black platform (visible platform, 10 cm in diameter) was placed in the pool with its top surface 0.5 cm above the water level. The mouse was allowed to find the platform to escape from the water. When the mouse could not escape within 60 s, it was picked up and placed on the platform. Training trials were performed on days 1 and 2. A circular transparent platform (invisible platform, 10 cm in diameter) was placed as in Fig. 1A, and its top surface was 0.5 cm below the water level. Three blocks of trials were performed daily for two consecutive days. Each block consisted of two 60-s tests. The mouse was allowed to swim freely for 60 s or until it arrived and mounted the platform. When the mouse found the platform within the 60 s, it was allowed to rest on the platform for 30 s. When it failed to locate and mount the platform within the 60 s, the mouse was forced to swim towards the platform and forced to take a rest for 30 s. It was then placed into a cage and allowed to groom freely for 5 min before another test was initiated. The interval of each block was 2 h. On the following day (day 3), the platform was removed and the mouse was made to search for the platform as a memory retention test. The number of crossing the imaginary platform within 60 s was recorded. LPS was injected 6 h prior to the first test on days 1 and 2.

Spontaneous alternation Y-maze test: The behavioral experiment was conducted in a Y-shaped maze (Fig. 1B). The three arms were each 395 mm in length and 120 mm in height and were separated by angles of 120°. A mouse was placed in the center of the apparatus and was allowed to explore the maze for 8 min. Arm choices were manually recorded. Any three consecutive choices of three different arms were counted as a correct choice. The ratio of correct choice was determined by dividing the total number of alternations by the total number of choices minus 2. LPS was injected 6 h before the test.

Passive avoidance test

Step-through test: The step-through test was performed according to the method used in our laboratory (19). The apparatus (Model PA-M1; O'hara & Co., Ltd., Tokyo) consisted of a lit and a dark compartment with electrifiable grid floor. The two compartments were separated by a black partition with a semi-circular doorway in the center. For the learning trial (day 1), a mouse was placed in the lit compartment and the latency before entering the dark compartment was recorded. When the mouse entered into the dark compartment and crossed an infrared beam placed 5 cm from the border, it received a 36 V AC foot-shock until it returned to the lit compartment. The mouse that received the shock was removed immediately so that it did not reenter the dark compartment. The testing trial (day 2) was performed 24 h later. The mouse was put into the lit compartment again and the time before entering the dark compartment was recorded as a step-through latency. When the mouse did not enter the dark compartment within 600 s, the test was terminated and a latency of 600 s was recorded. LPS was injected 6 h before the learning trial (day 1).

Grip power test

Grip power was measured 6 h after peripheral administration of LPS (MK-380; Muromachi Kikai Co., Ltd., Tokyo). Mice were put on the fence and pulled back slowly. The point of power which mice released the fence was determined as the grip power.

Pain threshold test

Threshold of pain was measured 6 h after peripheral

administration of LPS (MK-360; Muromachi Kikai Co., Ltd.). Mice were put in the chamber equipped with the stainless steel grid floor through which an electric shock was given. The intensity of an electric shock was increased gradually and the point at which mice began to vocalize was determined as the threshold of pain.

Statistical analyses

All data are expressed as means ± S.E.M. Statistical significance was evaluated with an analysis of variance followed by Tukey's multiple range test (body weight, grip toner, pain threshold, water maze test and Y-maze test) or Mann-Whitney test (step-through test). Differences were considered significant if $P<0.05$.

RESULTS

Physiological parameters

Peripheral administration of LPS (400 – 800 μg/kg) did not affect the body weight and grip tone of mice, however, high doses of LPS (2 and 10 mg/kg) significantly decreased the body weight (Fig. 2: A and B). LPS slightly decreased the pain threshold in a dose-dependent manner (Fig. 2C).

Spatial learning tests

Morris-type water maze test: Free swimming for 60 s on the day before the start of the experiment was conducted to allow the mice to become accustomed to water. Both LPS-injected and control mice swam well with the characteristic swimming posture. In the visible platform task on day 0, there was no significant difference in the escaping latency between the LPS-injected group and control group (data not shown), suggesting that LPS treatment did not cause sensorimotor disturbances. In the invisible (submerged) platform task on days 1 and 2, the LPS-injected group took a longer time to reach the platform than control group ($F(1,60) = 4.80801$, $P<0.05$ vs control), although the LPS-treated group arrived at the platform at the same time as the control group in the final trail (Fig. 3A). Memory retention of the platform location was assessed in the no platform task on day 3. Mice in both groups crossed the

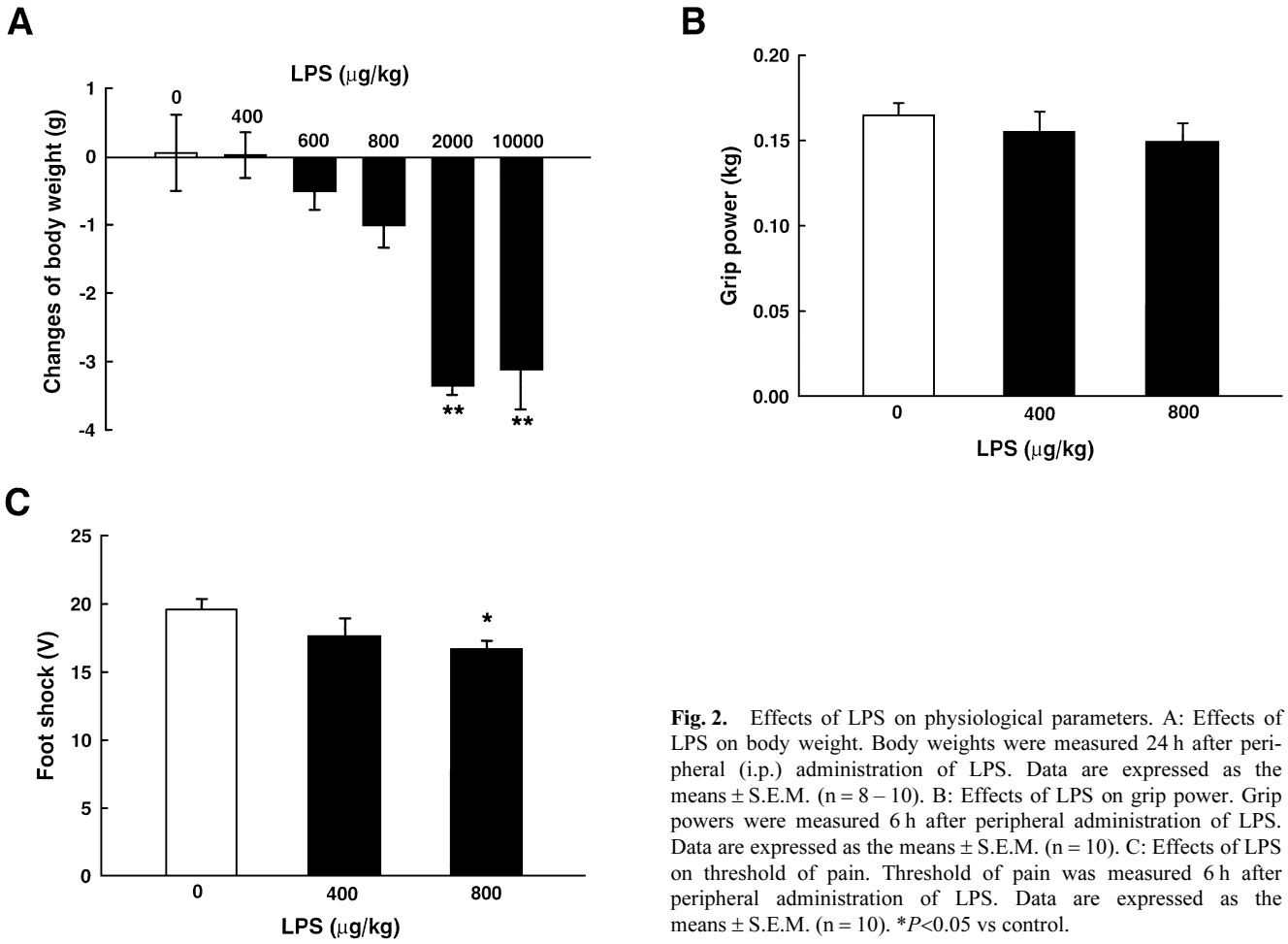

Fig. 2. Effects of LPS on physiological parameters. A: Effects of LPS on body weight. Body weights were measured 24 h after peripheral (i.p.) administration of LPS. Data are expressed as the means ± S.E.M. (n = 8 – 10). B: Effects of LPS on grip power. Grip powers were measured 6 h after peripheral administration of LPS. Data are expressed as the means ± S.E.M. (n = 10). C: Effects of LPS on threshold of pain. Threshold of pain was measured 6 h after peripheral administration of LPS. Data are expressed as the means ± S.E.M. (n = 10). *$P<0.05$ vs control.

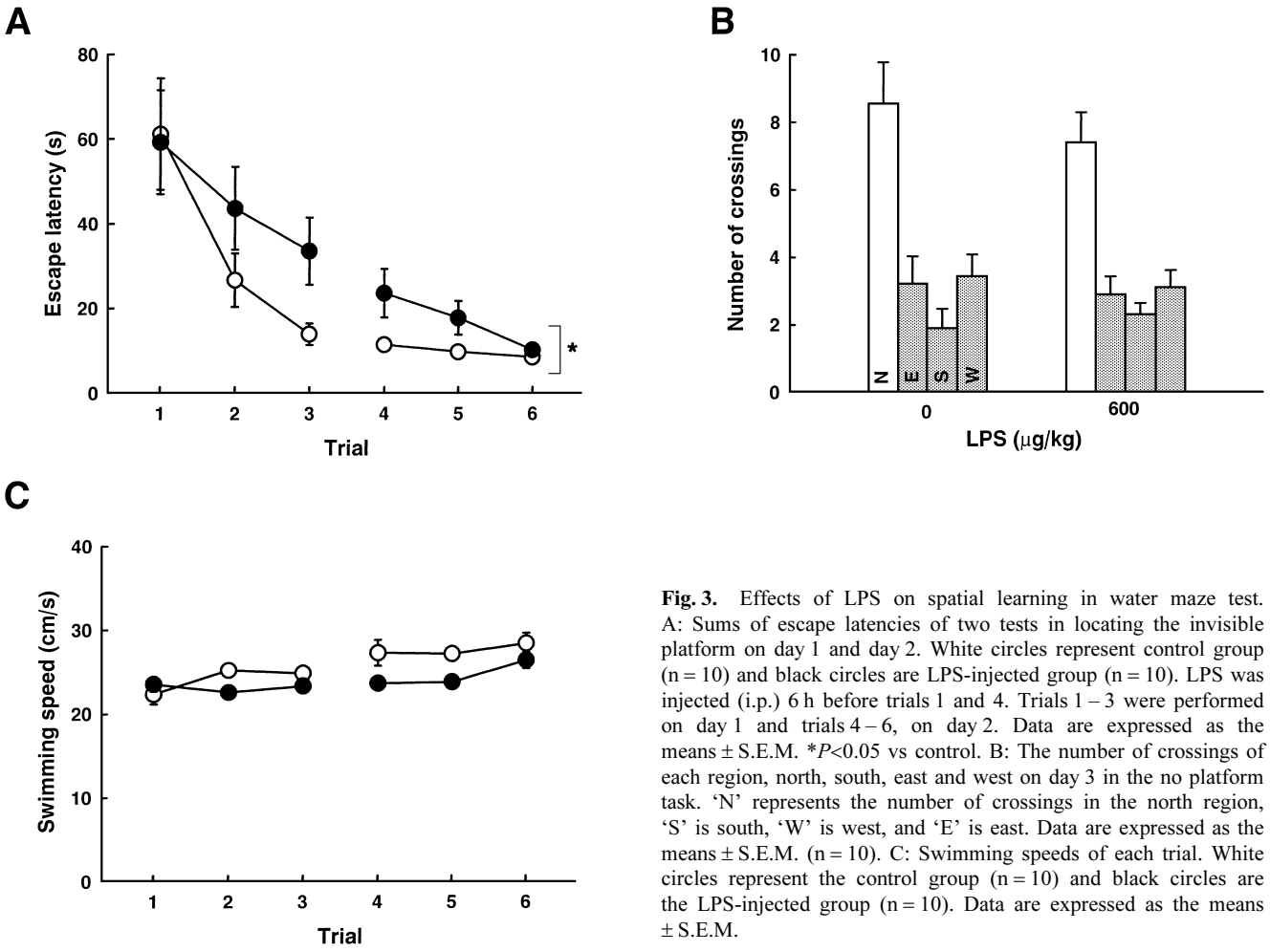

Fig. 3. Effects of LPS on spatial learning in water maze test. A: Sums of escape latencies of two tests in locating the invisible platform on day 1 and day 2. White circles represent control group (n = 10) and black circles are LPS-injected group (n = 10). LPS was injected (i.p.) 6 h before trials 1 and 4. Trials 1 – 3 were performed on day 1 and trials 4 – 6, on day 2. Data are expressed as the means ± S.E.M. **P*<0.05 vs control. B: The number of crossings of each region, north, south, east and west on day 3 in the no platform task. 'N' represents the number of crossings in the north region, 'S' is south, 'W' is west, and 'E' is east. Data are expressed as the means ± S.E.M. (n = 10). C: Swimming speeds of each trial. White circles represent the control group (n = 10) and black circles are the LPS-injected group (n = 10). Data are expressed as the means ± S.E.M.

platform location in the pool more frequently than the other areas, suggesting that they remembered the location (Fig. 3B). LPS treatment did not affect the swimming speed in all trails (Fig. 3C).

Spontaneous alternation Y-maze test: Mice were placed in the center of the Y-shaped maze, and their behavior was observed for 8 min. There was no difference in the number of arm choices or any preference for a particular arm after the LPS administration, suggesting that the endotoxin did not affect the motor activities (Fig. 4B). LPS treatment significantly decreased the ratio of correct response compared to the control in a dose-dependent manner (P<0.05 at 800 μg/kg of LPS-injected vs control) (Fig. 4A).

Passive avoidance test

Step-through test: Mice of all groups entered the dark compartment in the learning trial (day 1). In the testing trial (day 2), while almost all mice in the control group (9/10) did not enter the dark compartment within 600 s, mice in the LPS-treated group (4/10 at 400 μ/kg, 5/10 at 800 μg /kg) entered the dark compartment (Fig. 5).

DISCUSSION

We showed here, for the first time, that peripheral administration of LPS decreased the spatial learning performances in mice. The doses of LPS used in the learning tests were 400 – 800 μg/kg. These doses, in the present study, did not affect body weight, grip power, swimming speed and motor activity, suggesting that decreased performances in the learning tasks were due to the deterioration of learning abilities.

The Morris' water maze test was employed to examine a spatial learning performance in which mice were trained to escape from water by swimming to a submerged platform (20). Mice in the LPS-injected group took a longer time to reach the platform than those in the control group, suggesting that certain forms of associative memory were disturbed by administration of LPS. However, in the transfer test 24 h after the training tests, both the LPS-

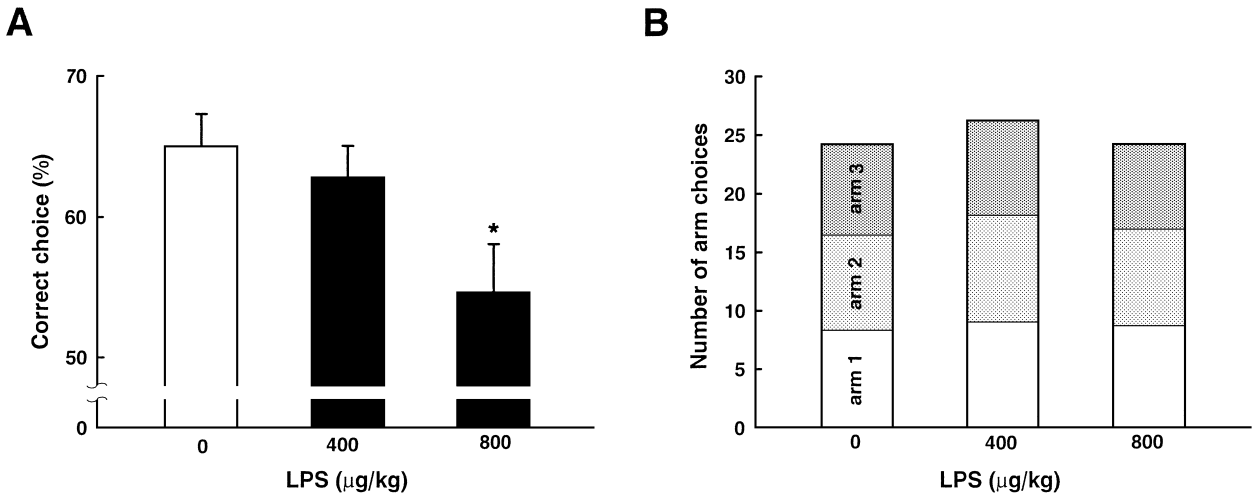

Fig. 4. Effects of LPS on spatial learning in the Y-maze test. A: The percentages of correct choices. LPS was injected (i.p.) 6 h before the test. Data are expressed as the means ± S.E.M. (n = 10). *P<0.05 vs control. B: The number of arm choices. White part represents the number of arm 1, light gray is arm 2, and dark gray is arm 3. Data are expressed as the means (n = 10).

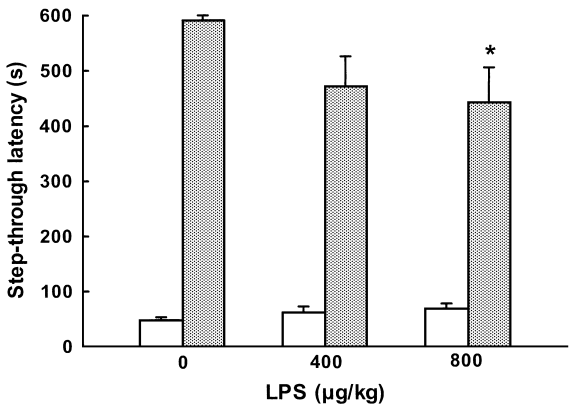

Fig. 5. Effects of LPS on learning in step-through type passive avoidance test. LPS was injected (i.p.) 6 h before the learning trial (day 1). White bars represent the step-through latency to the dark compartment in the learning trial (day 1), and gray bars represent that in the testing trial (day 3). Data are expressed as the means ± S.E.M. (n = 10). *P<0.05 vs control.

injected group and control group explored the correct position predominantly. According to this water maze test, it was shown that LPS treatment disturbed the memory acquisition, but not memory retention, of spatial learning. One explanation for this deterioration of spatial learning ability may be the increase of cytokines in the hippocampus. It is known that spatial learning and memory require integrative control functions of the hippocampus (12). These doses of LPS were sufficient to stimulate the immune system (e.g., IL-1β or TNF-α). In fact, injection of IL-1β impairs spatial navigation learning (21). It would be interesting to note the finding that IL-1 receptor antagonist prevented an impairment in contextual fear caused by intraperitoneal LPS administration (22).

For further investigation, we observed the effect of LPS on the spontaneous alternation Y-maze test. Although both water maze test and Y-maze test are classified as a spatial learning task, the former test was conducted using reference memory, while the latter was performed based on working memory (23). The memory component in the Y-maze task is that the mouse must remember which arm was most recently visited in order to alternate the arm choice. There were no differences between the three groups in the number of arm choice; however, LPS-injected mice decreased the ratio of correct response in a dose-dependent manner. Accordingly, the obtained results indicated that LPS decreased working memory as well as reference memory. Therefore LPS can be regarded as inducing deterioration of spatial learning abilities.

To examine further the effects of LPS on learning performance, we tested the step-through type passive avoidance task, an index of learning and memory of aversive experience (18). In the learning trial (day 1), there were no differences between the LPS-injected group and control group in the step-through latency, indicating that LPS treatment did not affect the motivation to the dark compartment. In the testing trial (day 2), the step-through latency of the LPS-injected group was slightly shorter than that of the controls. Therefore administration of LPS impaired certain forms of memory also in this task.

We examined the behavioral changes in mice at 6 h following the peripheral LPS administration, since the grip tone, swimming speed and motor activity were not altered during this time period. Furthermore intraperitoneal injection of LPS in rats was shown to increase the concentrations of norepinephrine, dopamine, serotonin and their

metabolites in the hypothalamic paraventricular nucleus measured by HPLC 5 h after the treatment (16). These changes were completely canceled by a concomitant treatment with an interleukin-1 receptor antagonist (16). More interestingly, intraperitoneal administration of LPS in freely moving rats increased extracellular concentrations of serotonin, noradrenaline, and their metabolites in the dorsal hippocampus as revealed by the in vivo microdialysis analysis (17). Although these biochemical changes reached maximal levels at 30 – 180 min depending on the neurotransmitter, the observed increase lasted for more than 6 h after the single LPS administration. In addition these changes were partly but significantly inhibited by indomethacin pretreatment, suggesting the involvement of the cyclo-oxygenase pathway (17). Recent finding showed that an anti-inflammatory agent reduced the learning impairment in rats induced by chronic intracerebroventricular LPS infusion (24). Thus it would be interesting to examine whether anti-inflammatory agents ameliorate the learning deterioration in mice observed in the present study. As for pharmacological intervention, nitric oxide synthase inhibitor might be another candidate, since direct injection of LPS into rat cortex/hippocampus increased nitrite and nitrate, nitric oxide metabolites, levels in the brain dialysate at 6 – 8 h after the LPS administration (25). The paper also reported that bilateral injection of LPS induced significant spatial learning impairment as shown in the present study. They examined the behavior seven days after the LPS administration, when hippocampal neuronal loss was apparent. Since we evaluated the leaning performances in mice 6 h after the peripheral LPS administration, the observed impairment may not be due to gross pathological changes in hippocampus.

As mentioned above, peripheral administration of LPS impaired a battery of learning performances. However, it is debatable how LPS induced this deterioration of learning performances. Long-term potentiation (LTP) may account for this question. LTP in the hippocampus is a form of synaptic plasticity and is considered to be one of the cellular bases of learning and memory (12). While behavioral stresses modify hippocampal plasticity (26), whether LPS affects LTP or not still remains to be clarified. Understanding whether or how LPS modifies synaptic plasticity in the hippocampus and subsequent learning processes may provide new approaches for individuals whose cognitive performances are impaired under morbid conditions.

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