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SHORT COMMUNICATION

Preventive effects of multisensory rehabilitation on development of cognitive dysfunction following systemic inflammation in aged rats

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Abstract Systemic inflammation can trigger transient or longer-lasting cognitive impairments, particularly in elderly patients. However, its pathogenesis has not been sufficiently clarified. In this study, we explored the potential effects of multisensory rehabilitation on cognitive dysfunction following systemic inflammation using an animal model. Aged male Wister rats were randomly injected intraperitoneally with either saline (control) or lipopolysaccharide (LPS; 5 mg/kg). After injection, both groups of rats were randomly assigned to either of two housing conditions ($n = 8$ in each condition): a standard cage environment (SC group) or a multisensory early rehabilitation environment (ER group). Cognitive function was examined after 7 days in the assigned environmental condition using a novel object recognition test. In the SC group, the LPS-treated rats showed impaired cognitive function compared with the control animals. These memory deficits were positively correlated with the levels of both tumor necrosis factor (TNF)-α and interleukin (IL)-1β in the hippocampus. On the other hand, in the LPS-treated ER group, neither cognitive impairment nor an increase in hippocampal levels of both TNF-α and IL-1β was found. These results imply that early rehabilitation (ER) intervention may be effective in preventing cognitive dysfunction following systemic inflammation via its anti-neuroinflammatory effects.

Keywords Rehabilitation · Inflammation · Cognitive dysfunction · Aged rats

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Systemic acute inflammation secondary to infection and tissue injury is reported to induce various cognitive complications, e.g., delirium and postoperative cognitive dysfunction [1–3]. These inflammation-associated cognitive disorders commonly occur in elderly patients and are associated with a significant increase in the length of hospitalization, need for institutionalization, and long-term mortality [4, 5]. Although the pathogenic mechanisms of inflammation-induced cognitive dysfunction remain elusive, pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), are considered to play a major role in its development [6–8].

There is preclinical evidence that environmental enrichment (EE), enriched housing conditions for stimulating sensory-motor systems, can improve cognitive deficits in a variety of animal models of nervous system disorders [9]. Furthermore, increases in physical activity are reported to reduce both peripheral and neuroinflammation and may be a potential intervention to reduce the onset or progression of cognitive dysfunction [10]. Indeed, early and aggressive mobilization is currently recommended for ICU patients to reduce the incidence or duration of delirium [11]. However, the effects and underlying mechanisms of a combination of EE and physical activity on inflammation-induced cognitive dysfunction are still not well investigated. Therefore, we examined whether early

rehabilitation (ER), consisting of EE and facilitated physical activity, could prevent the development of working memory deficit in a systemic infection model of aged rats.

All procedures were approved by the Institutional Animal Care and Use Committee of the Kochi Medical School. Male Wistar rats aged 24–25 months and weighing 585–640 g were used in this study.

Animals received a single intraperitoneal (i.p.) injection of either 5 mg/kg LPS (diluted in 0.5 ml saline) or an equivalent volume of saline. The dose of lipopolysaccharide (LPS) was determined based on a previous study [12]. After injection, each treatment group of rats was randomly housed either in EE combined with multimodal sensory stimulation as an early rehabilitation model (ER group) or in a standard cage environment (SC group). We used eight rats in each group. The EE consisted of a large cage equipped with plastic toys, a tunnel, a ladder, a platform, nesting material, and a running wheel (Fig. 1a). These items were routinely rearranged during the experimental period. The multimodal sensory stimulations were conducted by a buzzer sound (167 Hz), a blinking light-emitting diode (LED) light (3 Hz), and vibration (60 dB) for 1 min using a multidigital tuner (JT-NT01; Notty, Tokyo, Japan) three times daily during the active phase. The standard environment consisted of a small cage with bedding but no exploratory objects. All rats were pair-housed continuously in their respective environments until cognitive testing.

Cognitive function was examined after 7 days in the assigned environmental condition, using a novel object recognition test as described previously [13]. Briefly, each rat was individually habituated to the test chamber without an object for 5 min on 3 consecutive days. The experimental apparatus consisted of a Plexiglas open-field box with an open top. Objects were made of plastic and differed in color and shape but were similar in size. On the day of testing, two identical objects were placed in the experimental chamber, and each rat was allowed to explore the objects for 5 min (training phase). After a 1-h retention period, the rat was placed back into the experimental chamber with a new set of objects containing one identical and one novel object (testing phase). The rat was again allowed to explore the objects for 5 min. All testing was video-recorded and videos were analyzed later by an experimenter who was blind to the study group. Object exploration was defined as time spent sniffing the object

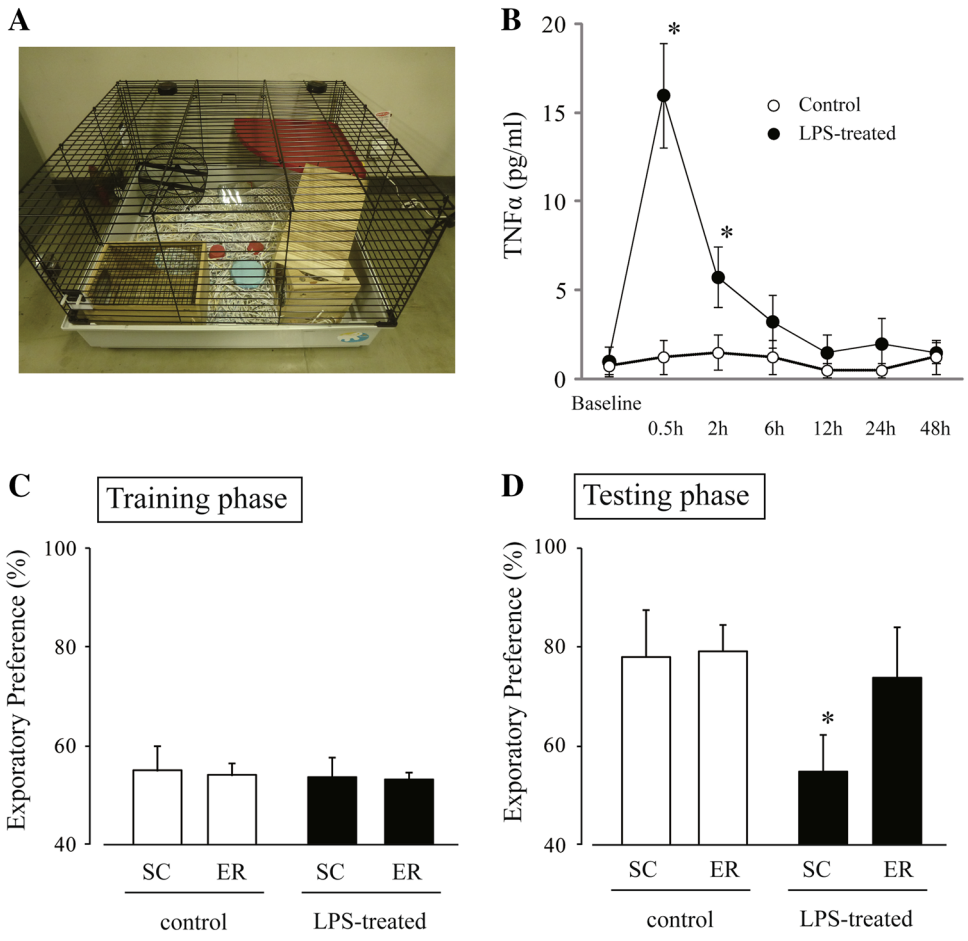

Fig. 1 Lipopolysaccharide (*LPS*)-induced cognitive deficits are prevented by multisensory rehabilitation in aged rats. **a** Environmental enrichment equipment used in the multisensory rehabilitation model. **b** Plasma levels of tumor necrosis factor (TNF)-α were measured before (baseline) and 0.5, 2, 6, 12, 24, and 48 h after intraperitoneal (i.p.) injection of LPS or vehicle (control). $*p < 0.05$ vs. baseline. Percentage of preference between two objects in the training phase (**c**) and in the testing phase (**d**) of the novel object recognition test performed 7 days after housing in a standard cage (*SC* group) or early rehabilitation condition (*ER* group) in saline (control)- or LPS-treated rats. Each *vertical bar* represents mean ± SD ($n = 8$ in each group). $*p < 0.05$ vs. control SC group

when the rat's nose was in contact with the object or within 1 cm from the object and the vibrissae were moving. Recognition memory is expressed as the ratio of time spent exploring either of the two objects during the training phase or the novel object during the testing phase over the total time spent exploring both objects.

After completion of the cognitive testing, all animals were killed by cervical decapitation under terminal anesthesia with inhaled isoflurane. The hippocampus was dissected and was stored at −80 °C until required for enzyme-linked immunosorbent assay (ELISA). In a separate sentinel experiment, blood samples were collected from the tail vein before and 0.5–48 h after injection to measure the serum levels of TNF-α (four rats/group). The levels of cytokines were measured using commercially available ELISA kits for rat IL-1β (ER2IL1B; Thermo Scientific) and TNF-α (438207; Biolegend).

All data are expressed as the mean ± standard deviation (SD). Differences between the data sets were evaluated by performing a repeated-measure one-way analysis of variance test, followed by Bonferroni post hoc tests. Results with $p < 0.05$ were considered statistically significant.

The single i.p. injection of LPS was well tolerated: all animals survived, and body weight gains did not differ between LPS-treated and control groups throughout the study. A slight increase of body temperature (<0.5 °C) was observed from 4 h after injection of LPS, but this returned to basal levels by 12 h. In a separate experiment, LPS-induced systemic inflammation in our model was confirmed by transient elevated serum levels of TNF-α, peaking at 0.5 h and returning to baseline levels by 6 h, after LPS injection (Fig. 1b).

One week after the injection of LPS, effects of ER on hippocampal-mediated working memory were assessed by a novel object recognition test. During the training phase, there was no biased exploratory preference for either one of the two objects among all groups (Fig. 1c). During the testing phase, in the SC group, the control rats spent more time exploring the novel object than the familiar object, whereas the LPS-treated rats exhibited significantly impaired novel object recognition performance as shown by the similar amount of time spent in exploring the two objects (Fig. 1d). However, such impairment was not observed in the LPS-treated ER group. Total exploration time in both training and testing phases did not differ among the groups, indicating that task motivation and locomotor ability during testing were comparable in all groups.

Taking the control and LPS-treated rats in the SC group together, the novel object recognition performance in the testing phase was inversely correlated with the levels of both TNF-α and IL-1β in the hippocampus (Fig. 2a, b).

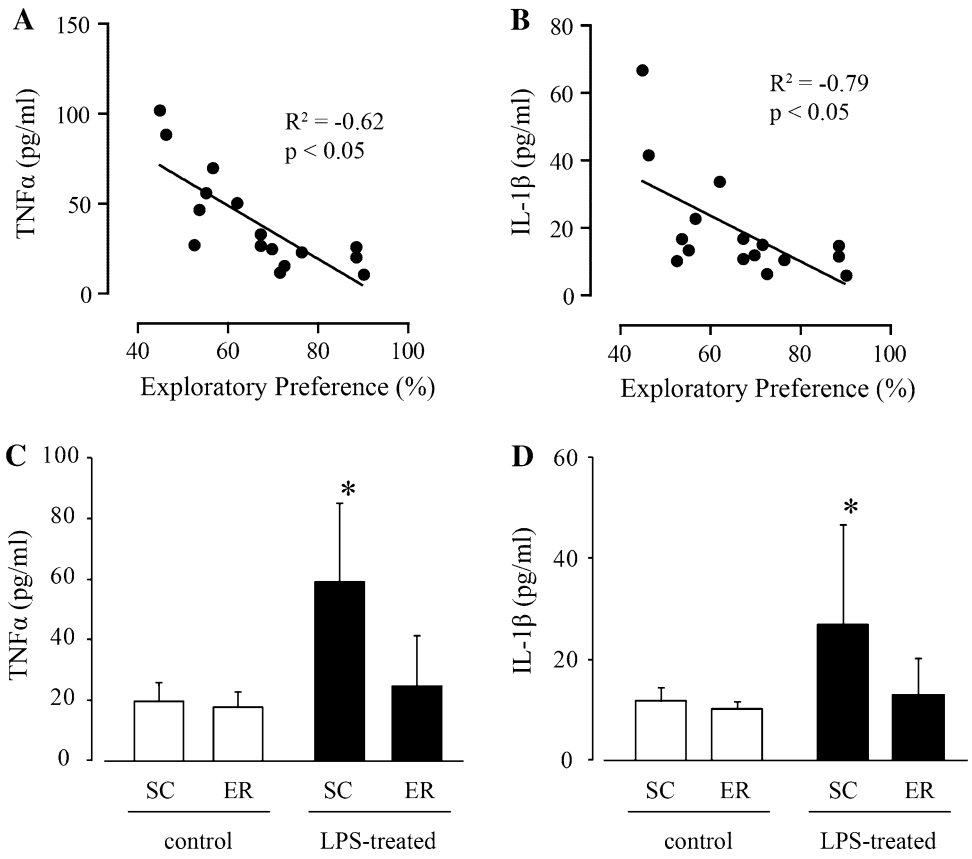

Fig. 2 Levels of cytokines in the hippocampus. Correlation of the levels of either TNF-α (**a**) or interleukin (IL)-1β (**b**) in the hippocampus with each donor rat's novel object recognition performance at the testing phase showed an inverse relationship. Average levels of TNF-α (**c**) and IL-1β (**d**) in each group are shown. Each *vertical bar* represents the mean ± SD ($n = 8$ in each group). *$p < 0.05$ vs. control SC group

This relationship suggests that neuroinflammation may play a pivotal role in cognitive deficits after systemic inflammation in aged rats. The average levels of hippocampal TNF-α and IL-1β in the LPS-treated SC group were significantly higher than those in the control SC and ER groups (Fig. 2c, d). However, the cytokine levels in the LPS-treated ER group were comparable with those of the control SC and ER groups.

The main finding of this study was that ER intervention for 7 days after i.p. injection of LPS prevents the development of LPS-induced cognitive dysfunction. Our results also indicated that the elevation of TNF-α and IL-1β in the hippocampus, observed in LPS-treated rats housed in an SC environment, was attenuated in those housed in an ER environment. Specifically, TNF-α is reported to reduce synaptic plasticity and contribute to the development of neurodegeneration [14]. In addition, IL-1β is known to inhibit long-term potentiation in the hippocampus and reduce performance on hippocampal-dependent memory tasks [14, 15]. Although it will be necessary to find out whether other pro-inflammatory cytokines also play a role, these findings imply that intervention with ER could mitigate LPS-induced cognitive impairment via the prevention of hippocampal neuroinflammation in aged animals.

Neuroinflammation characterized by increases in production of pro-inflammatory cytokines is a common pathogenesis of cognitive dysfunction associated with a variety of neurodegenerative diseases [6, 8]. Especially in the aged hippocampus, levels of pro-inflammatory cytokine have been found to be chronically elevated, and thus vulnerability to the detrimental effects of neuroinflammation is increased [16]. Indeed, advanced age was a consistent and well-established risk factor for cognitive deficits following critical incidents in most studies addressing risk [11, 17]. Although there is currently no available treatment for hippocampal neuroinflammation, our results suggest that early multisensory rehabilitation may be a noninvasive and nonpharmacological strategy for attenuating neuroinflammation and increasing plasticity and resiliency in the aged hippocampus. Similar to our findings, in animal models of both traumatic brain injury and stroke, early intervention with an enriched environment results in the reduction of pro-inflammatory cytokines within the site of injury [18, 19]. Moreover, our findings may partially underlie the recent clinical observations demonstrating that early initiation of physical therapy coupled with daily interruption of sedation could improve functional outcomes, including the incidence and duration of delirium [20].

The duration of a rehabilitation program may be a key factor to improve the cognitive outcome. However, currently there is no standardized rehabilitation protocol in animals, making it difficult to compare with laboratory results. Most studies on EE that employ protocols of duration have a short-term range, e.g., 1 week up to 2–3 months [21]. In addition, a previous study showed that multimodal sensory stimulation combined with EE promotes cognitive recovery in an animal model of traumatic brain injury [22]. Thus, because both EE and multimodal sensory stimulation were included, the ER intervention used in this study could provide beneficial effects in a short 1-week period.

Consistent with previous findings [23], our results show that acute systemic inflammation can trigger long-lasting neuroinflammation. Although it remains unclear how systemic inflammation interacts with the central nervous system, a recent study indicated that peripheral inflammation can indirectly produce brain cytokines, most likely via neuron–glial interaction within the brain [24, 25]. Especially in aging processes, some microglia develop a more inflammatory phenotype known as "microglial priming" [26]. These primed microglia are more responsive to peripheral signals and may induce long-lasting neuroinflammation. On the other hand, both physical activity and EE intervention are reported to increase brain-derived neurotrophic factor (BDNF) levels in the hippocampus [9]. Recently, several studies proved that BDNF exerts anti-neuroinflammatory effects during experimental brain damage [27, 28]. It is therefore possible that intervention of ER induces upregulation in hippocampal BDNF, which prevents the formation of long-lasting neuroinflammation. However, further studies are needed to evaluate this hypothesis.

In conclusion, our findings demonstrate that ER intervention prevents the development of cognitive dysfunction, as well as the elevation of TNF-α and IL-1β in the hippocampus, following i.p. injection of LPS in aged rats.

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