

【Report】

## Reduction in anxiety-like behavior in environmental enrichment

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Key words: enriched environment, wheel running activity, locomotor activity, anxiety.

### Introduction

Environmental enrichment (EE) involves housing conditions that facilitate enhanced sensory, cognitive, and motor stimulation<sup>9)</sup>. Previous studies have suggested that EE can enhance exploration, social interaction, and physical exercise in animals, leading to improvements in cognitive function, depression, and anxiety-like behavior<sup>1-13)</sup>. Based on these findings, it is widely accepted that EE conditions have neuro-protective effects on a range of brain functions.

The beneficial effects of EE on anxiety-like behavior is thought to be due to increases in locomotor activity. However, given that EE usually involves wheel running, it is still unclear whether reduction in anxiety-like behavior in EE is ascribed to increases in wheel running activity and/or locomotor activity except for wheel running.

The present study examined the effects of wheel running activity and locomotor activity in the absence of wheel running on anxiety-like behavior. Wheel running activity and locomotor activity of each rat was recorded using a recently developed device. The purpose of the present study was to determine the factors that reduce anxiety-like behavior in EE.

### Materials and Methods

#### A. Experimental animals and environmental housing conditions

All animal care and protocols were approved by the Physical Fitness Research Institute, Meiji Yasuda Life Foundation of Health and Welfare Animal Care and Use Committee (Approval number: 2014002). Male Wistar rats (4 weeks of age; Japan SLC, Shizuoka, Japan) were housed in a temperature-controlled room ( $22 \pm 2$  °C) with a 12 : 12-h light-dark cycle, and received standard rat chow and water *ad libitum*. The present EE consisted of running wheel, slope, tunnel, and small hut. Rats were divided into four different housing groups (standard environment: SE, only running wheel group: EE-W, EE without running wheel (only slope, tunnel, and hut) group: EE-S, and EE,  $n = 7-8$ , each). In the SE group, rats were housed in groups of 2 rats per cage in standard laboratory cages (length  $\times$  width  $\times$  height: 60  $\times$  40  $\times$  25 cm). In the EE group, rats were housed in groups of 2 per cage in large cages (60  $\times$  40  $\times$  40 cm).

#### B. Measurement of locomotive activity

Locomotor activity was continuously recorded using three-axis accelerometers (Nano-Tag: 15  $\times$  14.2  $\times$  7.1 mm, 2.5g, Kissei Comtec Co. Ltd., Nagano, Japan). The accelerometers were subcutaneously

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implanted in the back under anesthesia. The accelerometer counted locomotor activities above the threshold that was determined based on the preliminary experiments. In this study, we determined movements during feeding behavior as the threshold. This allowed to detect all kinds of movements in the cage as locomotor activity.

### C. Anxiety-like behavior measurement

After exposure to each environment for 6 weeks, the animals were submitted to the light-dark exploration test to assess anxiety-like behavior.

### D. Statistics

All experimental data are expressed as mean  $\pm$  standard deviation. Comparisons were performed using one-way analysis of variance followed by Bonferroni multiple comparisons. The level of significance was set at  $P < 0.05$ .

## Results

### A. Locomotor activity

Locomotor activity was higher during the dark period in the EE group ( $28260 \pm 12705$  frequency/day) compared with SE ( $19757 \pm 9964$ ,  $P = 0.02$ ) and EE-S ( $15703 \pm 6510$ ,  $P < 0.001$ ) groups. Locomotor activity was higher during the dark period in the EE-W ( $23182 \pm 9730$ ) group compared with EE-S group ( $P = 0.02$ ).

### B. Light-dark exploration test

The light-dark exploration test indicated that time spent in the light compartment was significantly longer in the EE-W group ( $145 \pm 65$  sec,  $P = 0.05$ ), EE-S group ( $163 \pm 63$  sec,  $P = 0.01$ ), and the EE ( $156 \pm 24$  sec,  $P = 0.01$ ) groups as compared with the SE group ( $68 \pm 30$  sec).

## Discussion

As predicted, anxiety-like behavior reduced in the EE group as compared with the SE group. Anxiety-like behavior also reduced in the EE-W group. This indicates that wheel running activity contributed to reduce anxiety-like behavior. Locomotor activity

reduced in the EE-S group as compared with other EE groups (EE-W and EE), which suggests that wheel running plays a key role in increase in locomotor activity in the EE condition. Nevertheless, anxiety-like behavior reduced in the EE-S group as compared with the SE group. Locomotor activity was comparable between SE and EE-S groups. Hence, these results suggest that reduction in anxiety-like behavior in the EE-S group is not primarily due to locomotor activity, but to enriched environment *per se*. Collectively, the present study suggests that both wheel running activity and locomotor activity in the EE condition reduce anxiety-like behavior. Locomotor activity in the SE may be less effective to reduce anxiety-like behavior as compared with the EE condition despite same amount of locomotor activity.

In conclusion, both wheel running activity and locomotor activity without wheel running reduce anxiety-like behavior in the EE. The EE seems to be helpful to reduce anxiety-like behavior.

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