体力研究 BULLETIN OF THE PHYSICAL FITNESS RESEARCH INSTITUTE
No.116 pp.29～32 Apr., 2018

【Report】

# Does environmental enrichment increase locomotor activity in rats? Evidence from an implanted sensor device

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Key words: environmental enrichment, locomotive activity, anxiety.

## Introduction

Environmental enrichment（EE）involves housing conditions that facilitate enhanced sensory, cognitive and motor stimulation relative to standard housing conditions[7)]. Previous studies have suggested that EE can enhance exploration, social interaction, cognitive function and physical exercise in animals, leading to improvements in depression and anxiety-like behavior[1,2,4,5,8)]. Based on these findings, it is widely accepted that EE conditions have neuroprotective effects on a range of brain functions.

The beneficial effects of EE on brain function are, at least in part, associated with increases in locomotor activity. However, to the best of our knowledge, actual locomotor activity has not been directly examined using an EE paradigm because of the technical difficulties involved in housing rats in groups. In the present study, we used a recently developed device to quantitatively assess locomotor activity in rats. With this device, it is feasible to assess locomotor activity accurately, even when rats are housed in groups. Hence, we measured locomotor activity of each rat housed under EE conditions during dark and light periods. Furthermore, because animals were exposed to motor stimulation in EE[7)], we expected that skeletal muscles may be affected（i.e., muscle hypertrophy）by EE.

The purpose of the current study was to assess actual locomotor activity of each rat in EE conditions using a small three-axis accelerometer. In addition, we also examined whether skeletal muscles exhibited hypertrophy when rats were housed in EE conditions.

## 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（6 weeks of age; Japan SLC, Shizuoka, Japan）were housed in a temperature-controlled room（22 ± 2 ℃）with a 12-h/12-h light/dark cycle, and received standard rat chow and water *ad libitum*. Rats were randomly assigned to standard environment（SE）and EE groups. In the SE group（n = 7）, rats were housed in groups of two rats per cage in standard laboratory cages（length × width × height: 40 × 25 × 20 cm）. In the EE group,（n = 7）rats were housed in groups of two rats/cage in large cages（60 × 40 × 40 cm）containing a slope, a small hut, three tunnels,

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and a running wheel. These housing supplements were moved to different locations within the cage every week. After 32 days of exposure in each group, the animals were anesthetized by isoflurane inhalation (2%). The tibialis anterior (TA), extensor digitorum longus (EDL), soleus (Sol), plantaris (Pla), and gastrocnemius (Gas) muscles, adrenal gland (AG), and thymus were removed and immediately weighted.

**B. Measurement of locomotive activity**

Locomotor activity was continuously recorded using three-axis accelerometers (Nano-Tag: 15 × 14.2 × 7.1 mm, 2.5 g, Kissei Comtec Co. Ltd., Nagano, Japan). The accelerometers were subcutaneously implanted in the back under anesthesia. The accelerometer counted the number of movements above the threshold that was determined based on the preliminary experiments. In this study, we determined movements during feeding behavior as the threshold, which allowed detection of movements in the cage as locomotor activity.

**C. Statistics**

All experimental data are expressed as mean ± standard deviation. Comparisons were performed using t-tests. The level of significance was set at $P < 0.05$.

## Results

**A. Skeletal muscle, adrenal gland, and thymus weights**

TA, Sol, and Pla muscle wet weights per body weight (BW) were greater in the EE group (TA: 1.56 ± 0.03 mg/g, Sol: 0.40 ± 0.02 mg/g, Pla: 0.93 ± 0.02 mg/g) compared with the SE group (TA: 1.50 ± 0.07 mg/g, Sol: 0.37 ± 0.02 mg/g, Pla: 0.90 ± 0.03 mg/g) ($P = 0.03$, $P = 0.02$, $P = 0.02$, respectively). In contrast, EDL and Gas muscle wet weight and per BW were no different between the EE and the SE (EDL: 0.40 ± 0.02 mg/g, Gas: 4.52 ± 0.21 mg/g) groups ($P = 0.45$, $P = 0.07$, respectively). There were no differences in AG and thymus weights between the EE (AG: 0.09 ± 0.01 mg/g, thymus: 1.27 ± 0.15 mg/g) and SE (AG: 0.08 ± 0.01 mg/g, thymus: 1.13 ± 0.12 mg/g) groups ($P = 0.22$, $P = 0.38$, respectively).

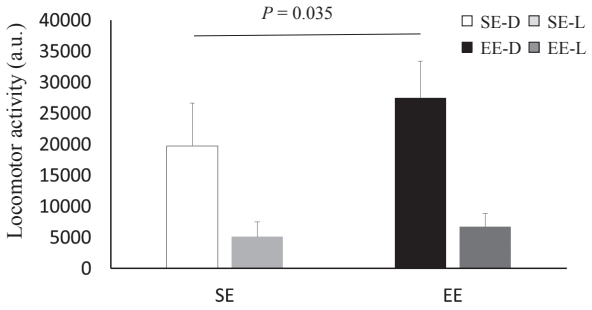


Figure 1. Locomotor activity.
Activity during the dark (D) or light (L) periods.
Values are expressed as mean ±SD.

**B. Locomotor activity in dark and light periods in SE and EE**

Figure 1 shows locomotor activity during dark and light periods in the EE and SE groups. Locomotor activity was greater in the EE group compared with the SE group during the dark period (EE: 28194 ± 6087 a.u., SE: 19757 ± 6909 a.u., $P = 0.03$). In contrast, locomotor activity during the light period was no different between the EE and SE groups (EE: 6704 ± 2313 a.u., SE: 5132 ± 2381 a.u., $P = 0.23$).

## Discussion

The major findings of this study were that: 1) locomotor activity was greater in the EE group than in the SE group; 2) skeletal muscle (TA, Sol, and Pla) hypertrophy was observed in the EE group; and 3) adrenal gland and thymus weights were not affected in the EE group. These results suggest that EE enhances locomotor activity and leads to muscle hypertrophy without inducing a physiological stress response in rats.

In previous studies, locomotor activity was indirectly assessed using an infrared sensor[6,9]. However, although these methods are useful when an animal is housed alone, they are not suitable for measurements when animals are housed in groups.

To the best of our knowledge, this is the first study to directly examine the effects of EE on locomotor

activity using an implanted accelerometer when rats are housed in groups. As expected, we observed greater locomotor activity in the EE group during the dark period. In contrast, there were no differences in locomotor activity during the light period between groups. These results clearly indicate that the present EE was effective for enhancing locomotor activity during the dark period.

In the present study, the TA, Sol, and Pla muscles exhibited hypertrophy 32 days after exposure to EE. Given the greater locomotor activity in the EE group, we can assume that muscle hypertrophy was caused by enhanced locomotor activity. Nevertheless, it remains unclear how enhanced locomotor activity induced skeletal muscle hypertrophy. This point should be further investigated in future studies. However, muscle hypertrophy was not observed in the EDL and Gas muscles[3)], indicating that the Sol and Pla muscles increased after resistance wheel running, while only the Sol muscle increased after free-spinning running. These findings suggest that the occurrence of muscle hypertrophy is dependent on the type of muscle activity. In the present study, the EE contained a slope, hut, tunnels, and a wheel. The present results suggest that motor stimulation induced by locomotor activity through these objects led to skeletal muscle hypertrophy. The differential effects of muscle hypertrophy indicate that muscle stimulation and resultant muscle activation might be specific to the TA, Sol, and Pla muscles.

Finally, the EE condition used in the present study did not affect adrenal gland and thymus weights, indicating that rats in the EE group did not exhibit physiological stress responses. The present results suggest that this type of EE may provide an appropriate model for inducing muscle hypertrophy without inducing stress responses.

## Conclusion

The current study examined locomotor activity among rats housed in EE conditions. The results revealed that locomotor activity was greater under EE during the dark period relative to standard housing conditions. The current findings also indicated that the EE used in the present study induced skeletal muscle hypertrophy in rats. These results suggest that enhanced locomotor activity in EE conditions induces muscle hypertrophy.

**Acknowledgments**

This study was supported by a grant from the Grant-in-Aid for Young Scientists（A）（JP16H05919）and a Grant-in-Aid for Exploratory Research（JP26560336）from JSPS KAKENHI.

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