体力研究 BULLETIN OF THE PHYSICAL FITNESS RESEARCH INSTITUTE
No.119 pp.9～12 Apr., 2021

【Report】

# Effects of different environmental enrichment on locomotor activity and skeletal muscle volume in rats

Mizuki Sudo[1)] and Soichi Ando[2)]

Key words: enriched environment, wheel running activity, locomotor activity, skeletal muscle.

## Introduction

Environmental enrichment (EE) involves housing conditions that facilitate enhancing sensory, cognitive and motor stimulation[1)]. The housing environment is enriched with a variety of objects, which include running wheels, puzzles (mazes, plastic tubes in different configurations), and accessories (toys, ropes, ladders, tunnels, hanging objects, house, ramps, and platforms) to stimulate their attention and engagement in the environment[2,3)]. Previous studies have suggested that EE can enhance cognition, visual-spatial attention, spatial memory, motor coordination, and ameliorate anxiety- and depressive-like behaviors in rodents[4-6)]. Based on these findings, it is widely accepted that EE conditions including wheel running have neuroprotective effects on a range of brain functions.

Recent studies have explicitly explored the effects of exercise (running or endurance exercise)-independent EE on brain function[7)]. These studies suggested that EE has the beneficial effects on brain function even if wheel running was not included[8,9)]. These findings led us to consider that wheel running in the EE may not play a key role in beneficial effects on the brain function in rats.

Skeletal muscles are essential for physical activity and exercise, and the decline in physical activity with aging has been shown to be primarily due to age-related muscle atrophy. The beneficial effects of EE on brain functions are thought to be due to increases in locomotor activity. However, it is still unclear whether skeletal muscle volumes in EE are affected by locomotor activity. Particularly, the effects of wheel running on skeletal muscle volumes should be further investigated. To test the effects of wheel running on skeletal muscle volume, wheel running needs to be isolated in the EE. Thus, in the present study, we compared the effects on skeletal muscle volumes among EE, EE without wheel running, and wheel running only conditions.

The purpose of this study was to examine the effects of different environmental enrichment on locomotor activity and skeletal muscle volume. We hypothesized that locomotor activity and/or motor stimulation in a variety of environmental enrichment in EE enhance muscle development.

## Materials and Methods

### A. Experimental animals and environmental housing conditions

Male Wistar rats (6 weeks of age; Japan SLC, Shizuoka, Japan) were housed in a temperature-con-

1) Physical Fitness Research Institute, Meiji Yasuda Life Foundation of Health and Welfare, Tokyo, Japan.
2) Department of Mechanical Engineering and Intelligent Systems, Control Systems Program, University of Electro-Communications, Tokyo, Japan.

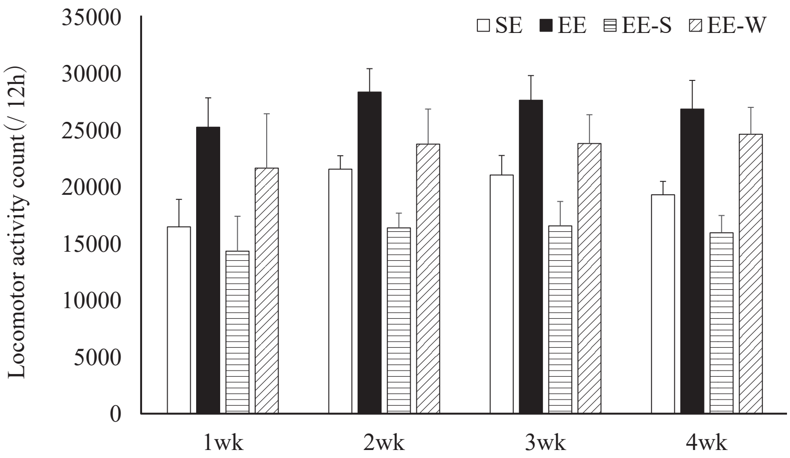


Figure 1. Locomotor activity counts during the dark period.
Values are expressed as mean ± SD.
SE: standard environment, EE: environmental enrichment, EE-S: without running wheel (only slope, tunnel, and hut) group, EE-W: only running wheel (without slope, tunnel, and hut) group.

trolled room (22 ± 2 ºC) 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 (without slope, tunnel, and hut) group: EE-W, 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 × width × height: 40 × 25 × 20 cm). In the EE groups, rats were housed in groups of 2 per cage in large cages (60 × 40 × 40 cm).

**B. Measurement of locomotor activity**

Locomotor activity in each rat was recorded using a recently developed three-axis accelerometers (Nano-Tag: 15 × 14.2 × 7.1 mm, 2.5 g, Kissei Comtec Co. Ltd., Nagano, Japan)[10,11]. This device is implanted subcutaneously in the back under anesthesia and records data individually[10,11]. The advantage of the devise is that specialized cages or devices are not required to measure locomotor activity in free-moving conditions. This means that activity data can be collected from multiple animals in the same cage, without constraining the testing location. The accelerometer counted 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. Soleus muscle volumes measurement**

After exposure to each environment for 30 days, soleus muscle was removed under anesthesia and immediately weighted.

**D. Ethical approvement**

All animal cares 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).

**E. Statistics**

All experimental data are expressed as mean ± standard deviation. Two-way analysis of variance (ANOVA) was performed with Week and Group as factors. Tukey's multiple comparisons test was used as a post-hoc test. Differences were considered statistically significant when $P < 0.05$.

## Results

**A. Locomotor activity**

Locomotor activity of rats was measured every day, the mean of those data was calculated per week in during the dark period (Figure 1). A two-way ANOVA found the statistically significant main effect of group ($F(3,27) = 7.137$, $P = 0.001$). The post-hoc analysis showed that locomotor activity of the EE group (1wk 25307 ± 2590, 2wk 28405 ± 2065, 3wk 27682 ± 2177, 4wk 26901 ± 2534 count/12h) was significantly higher than the SE group (1wk 16516 ± 2423, 2wk 21601 ± 1191, 3wk 21085 ± 1730, 4wk 19344 ± 1184 count/12h, $P = 0.043$). The EE-W group (1wk 21693 ± 4798, 2wk 23815 ± 3095, 3wk 23866 ± 2541, 4wk 24684 ± 2371 count/12h) showed significantly higher locomotor activity than the EE-S group (1wk 14369 ± 3084, 2wk 16419 ± 1312, 3wk 16595 ± 2159, 4wk 15990 ± 1531 count/12h, $P = 0.028$). Neither main effect of week nor interaction between group and week were observed.

**B. Soleus muscle volume**

Soleus muscle volume per body weight was greater in the EE-S (0.40 ± 0.03 mg/g) and EE-W groups (0.45 ± 0.03 mg/g) compared with the SE group (0.37 ± 0.02 mg/g, $P = 0.014$, $P < 0.001$, respectively). While the trend was same for the EE group (0.41 ± 0.02 mg/g, $P = 0.06$), the difference for soleus muscle volume between the SE group and EE group was not statistically significant in the post-hoc analysis.

## Discussion

The major findings of this study were that: 1) locomotor activity was greater in the EE group than in the SE group; 2) locomotor activity was lower in the EE-S (without running wheel) group as compared with the EE-W (with running wheel) group; 3) Soleus muscle volume was greater in the EE-W and the EE-S groups than the SE group. These results indicate that environmental enrichment condition, including running exercise, led to muscle hypertrophy in rats. The present findings suggest that locomotor activity and high frequency of low-intensity motor stimulation in environmental enrichment enhance muscle development in rats.

Locomotor activity reduced in the EE-S group as compared with the EE-W groups, which suggests that wheel running plays a key role in increase in locomotor activity in EE condition. The combination of voluntary wheel running, rearing, and walking with accessories may have important role for increasing daily locomotor activity. Indeed, the combined aerobic and resistance training circuit is effective for increasing daily activity in older woman[12]. Nevertheless, soleus muscle volume increased in all EE conditions as compared with the SE condition. Locomotor activity was comparable between the SE and EE-S groups.

Soleus muscle is vital for running, walking, and postural control. These results suggest that increased soleus muscle volume in the EE-S group is not only due to locomotor activity, but also muscle active pattern in enriched environment without running wheel.

A previous study has shown that low load resistance training led to similar muscle hypertrophy gains to high load resistance training[13]. In the present study, the EE-S group contained the slope, hut, and tunnels (not contained running wheel). The results of this study suggest that high frequency of low-intensity motor stimulation induced by locomotor activity through these settings may have led to skeletal muscle hypertrophy. However, future studies are needed to clarify the issue.

In conclusion, wheel running activity and locomotor activity without wheel running increased soleus muscle volume in the environment enrichment.

**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.

**References**

1) Nithianantharajah J, Hannan AJ. Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nature Reviews. Neuroscience. 2006; 7:

697-709.

2) Baumans V. Environmental enrichment for laboratory rodents and rabbits: requirements of rodents, rabbits, and research. ILAR Journal. 2005; 46: 162-70.

3) Singhal G, Jaehne EJ, Corrigan F, Baune BT. Cellular and molecular mechanisms of immunomodulation in the brain through environmental enrichment. Frontiers in Cellular Neuroscience. 2014; 8: 97.

4) Harati H, Majchrzak M, Cosquer B, Galani R, Kelche C, Cassel JC, Barbelivien A. Attention and memory in aged rats: Impact of lifelong environmental enrichment. Neurobiology of Aging. 2011; 32: 718-36.

5) Tees RC. The influences of sex, rearing environment, and neonatal choline dietary supplementation on spatial and nonspatial learning and memory in adult rats. Developmental Psychobiology. 1999; 35: 328-42.

6) Williams BM, Luo Y, Ward C, Redd K, Gibson R, Kuczaj SA, McCoy JG. Environmental enrichment: effects on spatial memory and hippocampal CREB immunoreactivity. Physiology and Behavior. 2001; 73: 649-58.

7) Birch AM, McGarry NB, Kelly AM. Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. Hippocampus. 2013; 23: 437-50.

8) Birch AM, Kelly AM. Lifelong environmental enrichment in the absence of exercise protects the brain from age-related cognitive decline. Neuropharmacology. 2019; 145: 59-74.

9) Singhal G, Morgan J, Jawahar MC, Corrigan F, Jaehne EJ, Toben C, Breen J, Pederson SM, Hannan AJ, Baune BT. The effects of short-term and long-term environmental enrichment on locomotion, mood-like behavior, cognition and hippocampal gene expression. Behavioural Brain Research. 2019; 368: 111917.

10) Sakai K, Ishikawa A, Mizuno Y, Maki T, Oda Y, Takahashi E. Simplified drug efficacy screening system for sleep-disorder drugs using non-human primates. Heliyon. 2020; 6: e03524.

11) Yoshizawa T, Shimada S, Takizawa Y, Makino T, Kanada Y, Ito Y, Ochiai T, Matsumoto K. Continuous measurement of locomotor activity during convalescence and acclimation in group-housed rats. Experimental Animals. 2019; 68: 277-83.

12) Obuchi S, Kawai H, Mitsutake S, Anzai S, Inomata T, Saito H, Tsuda H, Nakajima T. Effectiveness of combined aerobic and resistance training circuit on daily physical activity among community-dwelling older people. Japanese Journal of Physical Fitness and Sports Medicine. 2015; 64: 305-14.

13) Mitchell CJ, Churchward-Venne TA, West DW, Burd NA, Breen L, Baker SK, Phillips, SM. Resistance exercise load does not determine training-mediated hypertrophic gains in young men. Journal of Applied Physiology (1985). 2012; 113: 71-7.