Brief Communication

Effects of an enriched environment on rat skeletal muscles and plasma concentrations of noradrenalin and cortisol

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Introduction

An enriched environment has been suggested to enhance exploration, social interaction, cognitive function, and physical exercise, leading to improvements in depression and anxiety-like behavior^{5,6,9,10,14)}. An enriched environment can also lead to changes in neuroendocrine regulation^{4,11}. However, in contrast to the abundant evidence for beneficial effects of an enriched environment on brain function, little is known about how an enriched environment affects skeletal muscles and physiological response. An enriched environment comprises more complex housing with increased space, enhanced social interaction, and physical activity¹²⁾. Under an enriched environment, animals are probably exposed to many physiological stimuli. Hence, in the present study, we hypothesized that animals housed in an enriched environment would lead to alterations in skeletal muscle compared with those housed in a normal environment. To this end, in the present study, muscle wet weight was used to assess alterations in skeletal muscle under an enriched environment. In addition, blood noradrenalin and cortisol concentrations were measured to assess sympathetic nervous system activity and physiological and psychological stress in an enriched environment.

The purpose of the present study was to investigate whether muscle volumes are greater in animals housed in an enriched environment compared with those housed in a standard environment. We also investigated whether sympathetic nervous system activity and physiological and psychological stress are altered when animals are housed in an enriched environment.

Materials and Methods

A. Experimental animals and environmental housing conditions

All animal care and experimental 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 temperaturecontrolled room $(22 \pm 2^{\circ}C)$ with a 12-h/12-h light/dark cycle, and received standard rat chow and water *ad libitum*. The grouping of the animals in the different housing conditions was as follows: the standard environment (SE) group (n = 12) was housed at 2 rats/ cage in standard laboratory cages (length × width × height: $40 \times 25 \times 20$ cm); the enriched environment (EE) group (n = 12) was housed at 2 rats/cage in large

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Figure 1. An enriched environment cage (right) and the standard environment cage (left) in the present study.

cages $(60 \times 40 \times 40 \text{ cm})$ containing a slope, a small hut, three tunnels, and a running wheel (Figure 1). These housing supplements were moved to different locations within the cage every week.

B. Collection of blood and skeletal muscle samples

At the end of the 6-week exposure in each group, the animals were anesthetized by isoflurane inhalation (2%). Blood was withdrawn from the inferior vena cava in the morning. Briefly, blood (5 ml) was collected into tubes containing EDTA and kept on ice, before being centrifuged at $3000 \times g$ for 15 min at 4°C. The obtained plasma samples were frozen at -80° C until analysis.

The tibialis anterior (TA), extensor digitorum longus (EDL), soleus (Sol), plantaris (Pla) and gastroc nemius (Gas) muscles were removed and immediately weighed.

C. Analysis of plasma noradrenalin, dopamine, and cortisol concentrations

The plasma concentrations of noradrenalin and dopamine were determined using a high-performance liquid chromatography system (Shimadzu, Kyoto, Japan). The plasma cortisol concentrations were analyzed using an electrochemiluminescence immunoassay method (Hitachi, Tokyo, Japan).

D. Statistical analysis

All experimental data were expressed as mean \pm standard deviation. Comparisons were performed using a *t*-test. The level of significance was set at *P* < 0.05.

Results

A. Body weight

The body weights of the rats on the experimental day did not differ significantly between the two groups (SE: 254 ± 14 g; EE: 252 ± 11 g; P = 0.60).

B. Skeletal muscle wet weight

The absolute muscle wet weights and muscle wet

Skeletal Muscle	SE(n = 12)	EE(n=12)	Difference EE vs. SE, %	P value
TA, mg	396 ± 21	$421 \pm 14^*$	6.37	0.002
TA / BW, mg/g	4.56 ± 0.06	$1.67 \pm 0.05*$	7.51	< 0.001
EDL, mg	104 ± 5	104 ± 6	-0.72	0.736
EDL / BW, mg/g	0.41 ± 0.02	0.41 ± 0.02	0.24	0.894
Sol, mg	88.0 ± 6	$111 \pm 9*$	24.81	< 0.001
Sol / BW, mg/g	0.35 ± 0.02	$0.44 \pm 0.03*$	26.00	< 0.001
Pla, mg	219 ± 8	$239 \pm 15^*$	8.99	0.001
Pla / BW, mg/g	0.86 ± 0.03	$0.95 \pm 0.04*$	10.10	< 0.001
Gas, mg	1154 ± 60	$1212 \pm 58*$	4.50	0.025
Gas / BW, mg/g	4.55 ± 0.22	$4.81 \pm 0.08*$	6.00	0.001

Table 1. Skeletal muscle volume in SE and EE groups.

Values are expressed as mean \pm SD. n; no. of animals, SE; standard environment condition, EE; enriched environment condition, TA; tibialis anterior, EDL; extensor digitorum longus, Sol; soleus, Pla; plantaris, Gas; gastrocnemius, BW; body weight. * significantly different from SE group.





weights normalized by body weight (BW) are presented in Table 1. The TA, Sol, Pla, and Gas muscle wet weights per BW were greater in the EE group compared with the SE group. In particular, the increase in the Sol muscle wet weight per BW was the greatest and reached up to 26.0% in the EE group. In contrast, the EDL muscle wet weight and muscle wet weight per BW did not differ significantly between the two groups.

C. Plasma concentrations of noradrenalin, dopamine, and cortisol

The plasma noradrenalin concentration in the EE group (568 \pm 122 pg/ml) was significantly higher than that in the SE group (432 \pm 56.4 pg/ml) by 31.4% (*P* = 0.004; Figure 2A). There were no significant differences between the two groups in the plasma

dopamine concentrations (EE: 78.7 ± 34.1 pg/ml; SE: 56.1 ± 19.2 pg/ml; P = 0.069; Figure 2B) and plasma cortisol concentrations (EE: 1.00 ± 0.33 µg/ml; SE: 0.89 ± 0.40 µg/ml; P = 0.489; Figure 2C).

Discussion

The major findings of the present study were that: 1) the TA, Sol, Pla, and Gas muscle wet weights were greater in the EE group compared with the SE group, despite the finding that the body weights did not differ between the two groups; and 2) the plasma cortisol concentrations did not differ between the two groups, while the plasma noradrenalin concentration was greater in the EE group compared with the SE group. These findings suggest that the behavior of the rats in the enriched environment increased their skeletal muscle volumes without increasing physiological and/ or psychological stress.

Legerlotz et al.⁸⁾ examined the effects of voluntary wheel running with or without progressive resistance on rat skeletal muscles. They reported that the Sol and Pla muscles weight increased after resistance wheel running. In contrast, only the Sol muscle increased after free-spinning wheel running. These findings suggest that differences in resistance and muscle activation patterns during wheel running resulted in different muscle adaptations. In the present study, most skeletal muscles showed muscle hypertrophy in the EE group. It is noteworthy that the Sol muscle showed the greatest hypertrophy among all the muscles examined. In the EE group, the rats were housed in cages that contained a slope, a small hut, three tunnels, and a running wheel. Thus, the rats in the EE group probably experienced several physical activities that involved a variety of muscle activation patterns. Therefore, the present results suggest that the behavior in the EE group included muscle activation patterns that potentially increased muscle volume through hypertrophy. However, we did not assess metabolic adaptation associated with an enriched

environment. Increase in citrate synthase (CS) activity is known to reflect an improved aerobic metabolism in mitochondria of skeletal muscle⁷⁾. CS activity measurements will help to understand metabolic adaptation to the present enriched environment.

Peripheral noradrenalin is primarily released from sympathetic nerve endings, and reflects sympathetic nervous system activity¹³⁾. Cortisol is the primary hormone responsible for the stress response, which is regulated by the hypothalamic-pituitary-adrenal axis²). In the present study, the plasma noradrenalin concentration was greater in the EE group compared with the SE group. The standard plasma noradrenalin concentration is $100-300 \text{ pg/ml}^{3}$, and the present results exceeded this standard range. Under an enriched environment, it is plausible that rats were exposed to many physiological stimuli and that their sympathetic nervous system activity was intermittently enhanced. In contrast, the plasma cortisol concentrations did not differ between the EE and SE groups. Cortisol concentrations were close to those reported under the normal condition in the previous studies $^{1,15)}$. Hence, these results may suggest that the sympathetic nervous system activity was enhanced in the EE group without increasing physiological and/or psychological stress. The absence of differences in the cortisol concentrations between the two groups may suggest that the enriched environment employed in the present study is a suitable model for examining the effects of an enriched environment on the brain and skeletal muscle in rats.

In the present study, in the EE group, animals were housed in the same number as the SE group, which might lessen the beneficial effects of enriched environments. Hence, further studies are required to elucidate how skeletal muscle alters in the EE group when animals were housed in a greater number compared with the SE group. Moreover, cortisol was used to assess stress response in the present study. Given that the primary glucocorticoid in rodents is corticosterone, corticosterone should also be further investigated to assess stress response in the EE and SE groups. Finally, as noted in the Introduction, it is less clear how an enriched environment influences skeletal muscles and physiological response. Hence, it is important to accumulate empirical evidence to understand anatomical, metabolic, and circulatory adaptations in skeletal muscle as well as other organs after exposure to an enriched environment, which will provide a new insight into interaction between an enriched environment and body.

Conclusion

The enriched environment used in the present study appeared to increase rat skeletal muscle volumes without increasing physiological and/or psychological stress.

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References

- Akyazi, I. and Eraslan, E. (2014): Transmission of stress between cagemates: a study in rats. Physiol. Behav., 123, 114-118.
- Chrousos, G.P. (2009): Stress and disorders of the stress system. Nat. Rev. Endocrinol., 5, 374 – 381.
- Eldrup, E. and Richter, E.A. (2000): DOPA, dopamine, and DOPAC concentrations in the rat gastrointestinal tract decrease during fasting. Am. J. Physiol. Endocrinol. Metab., 279, E815 - E822.
- Fan, D., Li, J., Zheng, B., Hua, L., and Zuo, Z. (2016): Enriched environment attenuates surgery-induced impairment of learning, memory, and neurogenesis possibly by preserving BDNF expression. Mol. Neurobiol., 53, 344-354.
- 5) Hosseiny, S., Pietri, M., Petit-Paitel, A., Zarif, H., Heurteaux, C., Chabry, J., and Guyon, A. (2015): Differential neuronal plasticity in mouse hippocampus associated with various periods of enriched environment during postnatal development. Brain Struct. Funct., 220, 3435-3448.
- 6) Kempermann, G., Gast, D., and Gage, F.H. (2002):

Neuroplasticity in old age: sustained fivefold induction of hippocampal neurogenesis by long-term environmental enrichment. Ann. Neurol., **52**, 135 – 143.

- Lee, M.C., Okamoto, M., Liu, Y.F., Inoue, K., Matsui, T., Nogami, H., and Soya, H. (2012): Voluntary resistance running with short distance enhances spatial memory related to hippocampal BDNF signaling. J. Appl. Physiol. (1985), **113**, 1260 – 1266.
- Legerlotz, K., Elliott, B., Guillemin, B., and Smith, H. K. (2008): Voluntary resistance running wheel activity pattern and skeletal muscle growth in rats. Exp. Physiol., 93, 754 – 762.
- Li, G., Gan, Y., Fan, Y., Wu, Y., Lin, H., Song, Y., Cai, X., Yu, X., Pan, W., Yao, M., Gu, J., and Tu, H. (2015): Enriched environment inhibits mouse pancreatic cancer growth and down-regulates the expression of mitochondria-related genes in cancer cells. Sci. Rep., 5, 7856.
- 10) Mahati, K., Bhagya, V., Christofer, T., Sneha, A., and Shankaranarayana Rao, B.S. (2016): Enriched environment ameliorates depression-induced cognitive deficits and restores abnormal hippocampal synaptic plasticity.

Neurobiol. Learn. Mem., 134 Pt B, 379-391.

- Moncek, F., Duncko, R., Johansson, B.B., and Jezova, D.(2004): Effect of environmental enrichment on stress related systems in rats. J. Neuroendocrinol., 16, 423 – 431.
- Nithianantharajah, J. and Hannan, A.J. (2006): Enriched environments, experience-dependent plasticity and disorders of the nervous system. Nat. Rev. Neurosci., 7, 697-709.
- 13) Tank, A.W. and Lee Wong, D. (2015): Peripheral and central effects of circulating catecholamines. Compr. Physiol., 5, 1-15.
- 14) Tomiga, Y., Ito, A., Sudo, M., Ando, S., Maruyama, A., Nakashima, S., Kawanaka, K., Uehara, Y., Kiyonaga, A., Tanaka, H., and Higaki, Y. (2016): Effects of environmental enrichment in aged mice on anxiety-like behaviors and neuronal nitric oxide synthase expression in the brain. Biochem. Biophys. Res. Commun., 476, 635 – 640.
- 15) Velez-Marin, M., Hurtado Salazar, A., and Uribe-Velasquez, L.F. (2012): Plasma cortisol activity in rats under conditions of chronic stress supplemented with resveratrol. Colomb. Med. (Cali), 43, 221 – 225.