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Voluntary resistance running induces increased hippocampal neurogenesis in rats comparable to load-free running

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HIGHLIGHTS

- Voluntary resistance running (RWR) induced adult hippocampal neurogenesis (AHN).
- RWR-induced AHN is similar to that of voluntary running (WR).
- RWR produced higher work levels with shorter distances than did WR.
- There is no stress effect with RWR (at a maximum load of 30% of body weight).

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ABSTRACT

Recently, we reported that voluntary resistance wheel running with a resistance of 30% of body weight (RWR), which produces shorter distances but higher work levels, enhances spatial memory associated with hippocampal brain-derived neurotrophic factor (BDNF) signaling compared to wheel running without a load (WR) [17]. We thus hypothesized that RWR promotes adult hippocampal neurogenesis (AHN) as a neuronal substrate underlying this memory improvement. Here we used 10-week-old male Wistar rats divided randomly into sedentary (Sed), WR, and RWR groups. All rats were injected intraperitoneally with the thymidine analogue 5-Bromo-2′-deoxuridine (BrdU) for 3 consecutive days before wheel running. We found that even when the average running distance decreased by about half, the average work levels significantly increased in the RWR group, which caused muscular adaptation (oxidative capacity) for fast-twitch plantaris muscle without causing any negative stress effects. Additionally, immunohistochemistry revealed that the total BrdU-positive cells and newborn mature cells (BrdU/NeuN double-positive) in the dentate gyrus increased in both the WR and RWR groups. These results provide new evidence that RWR has beneficial effects on AHN comparable to WR, even with short running distances.

1. Introduction

The hippocampus is known to play a crucial role in cognitive functions, including learning and memory, and a number of studies have shown that newly generated neurons in the dentate gyrus (DG) contribute to these processes [7,12]. New neurons are produced continually in the subgranular zone (SGZ) of the DG throughout life [2,5], which refers to adult hippocampal neurogenesis (AHN). New neurons are derived from the proliferation of neural stem/progenitor cells and instructed by signaling to differentiate into neurons [6]. The newly generated neurons then migrate to the granule cell layer of the DG and integrate into the hippocampal circuitry, which may play a critical role in cognitive functions [7,10]. And AHN is known to be influenced by several factors, such as aging [15], stress [9], enriched environment [11], and physical activity [24,32]. In particular, exercise increases AHN, which is associated with hippocampus-dependent cognitive functions [31].

However, almost such studies have focused on exercise amounts (e.g., running distance), rather than exercise intensity. Voluntary wheel exercise is normally used to enhance hippocampal plasticity, since compelled treadmill exercise is considered a stressor to rodents. Stress leads to the inhibition of exercise-enhanced hippocampal adaptations such as AHN [22], though mild treadmill exercise does not [24]. However, with voluntary wheel running, the intensity, duration, and interval time of running cannot be precisely identified. Thus, the optimal exercise conditions for generating such beneficial effects for hippocampal plasticity are still under debate.

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Voluntary resistance wheel running (RWR) allows for a given load on a running wheel and increases work levels, which results in muscular adaptation in fast-twitch muscle without using physical and psychological stressors such as electrical shock and additional weight [3,8]. Indeed, we recently, reported that RWR, with shorter distances but higher work levels, which elicited muscular adaptation for fast-twitch plantaris muscle without causing negative stress effects and RWR enhances spatial memory associated with hippocampal brain-derived neurotrophic factor (BDNF) signaling [17]. Thus, RWR represents a successful model that enhances the effects of exercise on both muscular and hippocampal adaptations, even with short distances.

The exact nature of the underlying mechanism is still controversial, although a growing number of studies suggest a possible role of hippocampal BDNF [33]. BDNF is a key protein supporting the growth, development and survival of neurons and physical exercise increases hippocampal BDNF mRNA and protein [23,30] to promote AHN [16,26]. Alteration in BDNF signaling is apparently necessary for the effects of exercise on hippocampal plasticity in rodents, as blocking BDNF signaling inhibits exercise-enhanced learning and memory [33] and prevents exercise-induced in neurogenesis [18]. These findings lead us to assume that RWR may play beneficial effects on AHN.

To address this issue, we investigated whether resistance wheel running has potential effects on neurogenesis comparable with wheel running without a load.

2. Materials and methods

Animals. 10-week-old male Wistar rats (320–340 g; SEAS, Co., Ltd., Saitama, Japan) were randomly allocated to three groups: (1) housed in standard cages and used as non-active controls (Sed, $n = 6$), (2) wheel running with no resistance (WR, $n = 6$), and (3) housed in cages with resistance running wheels with an adjustable resistance (RWR, $n = 6$). All rats were individually housed and kept in a controlled environment with a 12 h–12 h light–dark cycle (lights on at 8:00 a.m.) and given ad libitum access to food and water. All the experiments were performed in accordance with protocols approved by the University of Tsukuba Animal Experiment Committee, based on the NIH Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1996).

Running-wheel apparatus and loading protocol. This RWR apparatus and its setup for resistance running were similar to those previously described [8,17]. Briefly, the rats of both running wheel groups (WR and RWR) were housed individually and had free access to a specially designed running wheel apparatus (diameter = 31.8 cm, width = 10 cm; Rat Analyzer KI-103, Aptec Inc., Kyoto, Japan) 24 h/day. The rats in the RWR group were exercised with minimum resistance (i.e., 4.5 g) for the first week, and then the resistance was progressively increased to reach 30% of their body weight during the 4 weeks of exercise.

BrdU administration and tissue preparation. In preparation for immunohistochemistry, all rats were injected intraperitoneally with the thymidine analogue of 5-Bromo-2′-deoxuridine (BrdU, 100 mg/kg, 1 injection per rat per day) for 3 consecutive days before wheel running [13]. After the final day of exercise, animals were deeply anesthetized (pentobarbital, 50 mg/kg, i.p.) and perfused transcardially with 0.9% saline followed by a solution containing 4% paraformaldehyde in 0.1 M phosphate-buffered (PB), pH 7.4. After perfusion, the brains were removed immediately from the skull and post-fixed in 4% paraformaldehyde in PB for 24 h. The brains were then cut coronally with a vibratome (Leica VT 1000 S, Nussloch, Germany) in 50 μm slices.

Sample collection. To measure stress levels, adrenals were excised and weighed, and blood was collected from the carotid artery for measuring plasma corticosterone levels. To measure muscle adaptations, the soleus and plantaris muscles were excised and weighed.

Measurement of skeletal muscle citrate synthase activity. The citrate synthase (CS) activity in the plantaris and soleus muscles was measured using a method described previously [17]. The CS activity in muscles was determined using the Citrate Synthase Assay Kit (Sigma, Saint Louis, MO, USA) following the manufacturer's instructions.

Radioimmunoassay. The plasma corticosterone levels were determined by the RIA method [21] by using [^{3}H]-corticosterone as a tracer. In brief, plasma was separated from trunk blood centrifugation at 3000 rpm for 10 min, and then stored at −80 °C before analysis. Samples and corticosterone standards were thawed at room temperature and added to tubes in triplicate. Then 0.1 ml of [^{3}H]-labeled corticosterone was added and mixed in each tube before incubating for 30 min at 37°C. Samples were then counted using a scintillation counter.

Immunohistochemistry. Double immunofluorescence staining for BrdU and NeuN was performed on one series of sections selected at random, as described previously [25]. Briefly, a series of one out of every ten sections was used for immunohistochemistry, and the samples were pretreated with 2 N HCl at 37 °C for 30 min to denature the DNA. Then the free-floating slices were incubated for 2 days at 4 °C with the primary antibodies diluted with 0.1 M PB containing 2% normal donkey serum (NDS) and 1% Triton X-100. The rat monoclonal anti-BrdU (1:500; AbD Serotec, UK) for newly proliferated cells and mouse monoclonal anti-NeuN (1:500; Chemicon, USA) for mature neurons were used as the primary antibodies. The slices were then incubated for 24 h at 4 °C with an appropriate secondary antibody: Cy3 donkey anti-rat (1:500; JACKSON, USA) or AMCA donkey anti-mouse (1:250; JACKSON, USA). The sections were mounted on gelatin-covered slides and analyzed with a Leica DMRB optical microscope (Leica, Bensheim, Germany).

Cell counting. An unbiased stereology method was used to quantify labeled cells. This was modified from a previously described method [20]. The number of BrdU-positive positive cells was counted through a 40× objective within the granular cell layer (GCL) and two cell diameters below the GCL. Next, a mean cell density for every animal was calculated. The volume of the granule cell layer of every animal was measured by means of the Cavalieri method [11] using Nissl sections. Finally, the total cell number for every population was calculated by multiplying the mean cell density by the granule cell layer volume animal by animal.

Statistical analysis. Average running distance and work levels on each of the experimental days were analyzed with repeated measures two-way ANOVA, followed by Bonferroni's multiple comparison test for post hoc analysis. The comparisons between different groups were analyzed with a one-way ANOVA followed by Tukey's multiple comparison tests for post hoc analysis. Statistical significant difference was evaluated at $p < 0.05$.

3. Results

Effects of RWR on exercise performance. Repeated measures with ANOVA showed significant day effects ($F(27, 270) = 4.62$, $p < 0.0001$) on daily running distance (Fig. 1A). The average running distance of the RWR group (470.1 m/day) decreased by about half that of the WR group (1022.8 m/day) ($p < 0.05$; Fig. 1B). Whereas, the average work levels analysis revealed significance for day ($F(27, 270) = 4.82$, $p < 0.0001$), group ($F(1, 10) = 8.02$, $p < 0.05$) and interaction between day and group ($F(27, 270) = 4.57$, $p < 0.0001$) effects (Fig. 1C). The average work levels significantly increased by about 7-fold in the RWR group (775.9 N m/kg b.w./day) compared to the WR group (108.3 N m/kg b.w./day) ($p < 0.01$; Fig. 1D).