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Running wheel exercises accelerate neuronal turnover in mouse dentate gyrus

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Abstract

Neurogenesis continues throughout adulthood in mouse dentate gyrus, and is influenced by environmental, endocrine, and pharmacological conditions. Although running wheel exercises have been reported to enhance neurogenesis, the effects on neuronal death in dentate gyrus are not well understood. The precise control of the production and elimination of neurons is thought to be important for the maintenance of a relatively constant number of neurons in the adult nervous system and for the regulation of adult brain function. We report here that running wheel exercises enhance the death of pre-existing neurons as well as neurogenesis in dentate gyrus. In addition, we analyzed mice lacking an NMDA receptor ε1 subunit, and found that the enhancement of the neuronal death by the exercises is suppressed in the ε1 subunit knockout mice. These results suggest that running wheel exercises accelerate neuronal turnover in mouse dentate gyrus, through the activation of NMDA receptors.

Keywords: Neurogenesis; Cell death; Turnover; Exercise; NMDA receptor; Hippocampus; Knockout mouse; BrdU

1. Introduction

Neurogenesis occurs in adult brains of different animal species, including human (Altman and Das, 1965; Eriksson et al., 1998; Gage, 2000). The neuronal progenitor cells are present in the subventricular zone and in hippocampal dentate gyrus. Adult neurogenesis leads to the addition of neurons to the hippocampus as its long-lasting constituents (Kempermann et al., 2003; Dayer et al., 2003), and is positively influenced by enriched environments (Kempermann et al., 1997; Nilsson et al., 1999; Young et al., 1999), running wheel exercises (van Praag et al., 1999b; Fabel et al., 2003; Kitamura et al., 2003; Farmer et al., 2004), hippocampus-dependent learning (Gould et al., 1999), and dietary restriction (Lee et al., 2000, 2002; Kitamura et al., 2006).

Conditional presenilin 1 gene knockout mice abolished neurogenesis and reduced extinction of contextual fear conditioning memory (Feng et al., 2001). The number of newly generated neurons was substantially reduced in the adult rat by treatment with a DNA methylating agent, methylazoxymethanol acetate (MAM), and this reduction impaired hippocampus-dependent trace conditioning (Shors et al., 2001). Irradiation of the hippocampus, which reduces hippocampal neurogenesis, abolishes the behavioral and proliferative effects of many different antidepressants in a novelty-suppressed feeding test (Santarelli et al., 2003). Moreover, running wheel exercises increased long-term potentiation in dentate gyrus and enhanced hippocampus-dependent learning (van Praag et al., 1999a; Farmer et al., 2004). These observations suggest that hippocampal neurogenesis may play an important role in the hippocampal function. However, it is not clear whether this stimuli-induced neurogenesis leads to a net increase in granule cell numbers and hippocampal volumes, or whether increased neurogenesis is matched with the increased neuronal elimination.

In this study, we analyzed the effects of running wheel exercises on cell death, cell proliferation and volume of dentate gyrus. Using the methods of chronic BrdU administration, we detected the dying neurons which have been pre-existing for at least 4 weeks in the dentate gyrus, and found that running wheel exercises enhanced the death of these pre-existing neurons in mouse dentate gyrus. We previously reported that running-induced cell proliferation and neurogenesis were suppressed in mice lacking an NMDA receptor ε1 subunit (Kitamura et al.,

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2003). Therefore, we also studied the neuronal death using the mice lacking the NMDA receptor ε1 subunit, and found that running-enhanced neuronal death was suppressed in the knockout mice. Our results suggest that running wheel exercises accelerate neuronal turnover in the dentate gyrus through the activation of NMDA receptors.

2. Methods

2.1. Animals

C57BL6 male mice (Kyudo, Fukuoka, Japan), and mutant mice lacking the NMDA receptor ε1 subunit, generated previously (Sakimura et al., 1995) and backcrossed with the C57BL6 mice to yield 99.99% C57BL6 genetic background (Kiyama et al., 1998), were used in this study. All animals were kept under temperature and light controlled conditions (23–25 °C, 12 h light/12 h dark cycle), received food and water ad libitum, and were maintained under the ethical regulations of the university.

2.2. Running wheel conditions

Mice were placed in the cage (width: 31 cm, height: 20 cm, depth: 24 cm) equipped with a running wheel (diameter: 16 cm, width: 8.5 cm). To analyze the running distance, an infrared sensor and an electronic preset counter (Keyence, Osaka, Japan) were used to count the number of wheel rotations.

2.3. BrdU methods

To evaluate the cell proliferation, BrdU (100 mg/kg, i.p., Sigma) was administered once and the mice were sacrificed 2 h after the BrdU injection. To label continuously proliferating progenitor cells, BrdU was given through drinking water (1.5 mg/ml) for 4 days or 4 weeks. Drinking BrdU-containing water for 4 weeks did not affect the density of the cells with nuclear condensation in dentate gyrus (naïve: 1.20 ± 0.18 cells/mm, BrdU: 1.06 ± 0.19 cells/mm, $P = 0.61$ by student's t-test, $n = 3$ animals).

2.4. Tissue preparation

All mice were fixed via transcardiac perfusion with 4% paraformaldehyde under anesthetization with ethyl carbamate (1.5 g/kg, i.p., Wako) and their brains were removed, post-fixed in 4% paraformaldehyde at 4 °C overnight, and transferred to a 30% sucrose solution. A cryostat was used to collect the coronal 40 μm sections though the hippocampus.

2.5. Histology

For BrdU immunohistochemistry, the sections were treated as follows to denature (Proteinase K 0.02 mg/ml for 10 min at room temperature, 2N HCl for 30 min at 37 °C, 0.1 M boric acid pH 8.5 for 10 min at room temperature), and sections were blocked with 5% normal donkey serum, 0.1% Triton X-100, 0.05% Tween 20/PBS for 1 h and incubated with the BrdU antibody (1:400, Immunologicals Direct). The secondary antibody was used anti-rat Cy3 (1:200, Jackson Immuno Research). Some sections were counterstained with SYTOX GREEN (1:4000, Invitrogen).

For NeuN immunohistochemistry, the sections were blocked with 5% normal donkey serum, 0.1% Triton X-100, 0.05% Tween 20/PBS for 1 h and incubated with the NeuN antibody (1:100, Chemicon). The secondary antibody used was anti-mouse FITC (1:200, Jackson Immuno Research) and counterstained with DAPI (1:10,000, 1 mg/ml, Sigma).

For immunofluorescent double-labeling of BrdU and NeuN, sections were treated as follows to denature: 2 h incubation in 50% formamide–2× SSC (0.3 M NaCl and 0.03 M sodium citrate) at 65 °C, 5 min rinse in 2× SSC, 30 min incubation in 2N HCl at 37 °C, and 10 min rinse in 0.1 M boric acid, pH 8.5. Sections were blocked with 5% normal donkey serum, 0.1% Triton X-100, 0.05% Tween 20/PBS for 1 h and incubated with the BrdU antibody (1:400, Immunologicals Direct) and NeuN antibody (1:100, Chemicon). The fluorescent secondary antibodies used were anti-rat Cy3, and anti-mouse FITC (1:200, Jackson Immuno Research).

To identify cells dying on the sections, DNA fragmentation was visualized by the terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) method (Promega), and counterstained with DAPI or SYTOX GREEN to identify the nuclei condensed cells.

2.6. Stereology

To count the BrdU-labeled cells, the number of BrdU-labeled cells in the subgranular zone (SGZ) and granule cell layer (GCL) was stereologically analyzed using the NIH image software. Our previous analyses showed that BrdU-labeled cells distribute uniformly throughout the hippocampus (Kitamura et al., 2003). In this study the slices were obtained successively from the anterior one-third position along the long axis of the hippocampus as follows. The brain was coronally sectioned from the anterior to posterior side. When the anterior end of the hippocampus came out, a section of 600 μm thickness was removed. Then 10 coronal sections of 40 μm thickness were taken out from there, and BrdU immunohistochemistry and DAPI staining to detect condensed nuclei cells were performed. For quantitative analysis, the linear densities of BrdU-labeled cells and cells with nuclear condensation [number of cells per unitary length (mm) of granule cell layer] were determined as described previously (Kitamura et al., 2003).

For the analysis of nuclei condensed cells and TUNEL positive cells, confocal microscopic analysis (LSM 510, Zeiss) was performed. To analyze the ratios of the numbers of dying cells in two regions (SGZ and GCL), 100 condensed nuclei cells from six animals were examined.

To evaluate the absolute volume of dentate gyrus, the 10–11 sections, 240 μm apart, containing dentate gyrus, were obtained. The dentate gyrus was counterstained with DAPI, and the area size of the dentate gyrus was stereologically evaluated (Adobe Photoshop program). The total volume of the dentate gyrus was calculated from the dentate area size and the thickness of the sections.

2.7. Statistics

All data were presented as mean S.E.M. Data were statistically analyzed by Student's t-test or by two ways ANOVA, using the Microsoft EXCEL program. The difference was considered significant when $P < 0.05$.

3. Results

3.1. Adult neurogenesis dose not contribute to the volumes of hippocampal dentate gyrus

We first examined if the continual neurogenesis may affect the volume of the dentate gyrus. For this purpose, total volumes of the dentate gyrus were stereologically determined in nuclei-stained hippocampal sections of C57BL6 mice. In the first experiment, the volumes of the dentate gyrus were determined at several ages (postnatal 2, 3, 6, and 24 weeks). The volumes of the dentate gyrus increased during the postnatal 2–3 weeks, but after postnatal 3 weeks, the volume of the dentate gyrus remained constant (Fig. 1A).

In the second experiment, we examined the effects of the running wheel exercises on the volumes of the dentate gyrus. Our previous report showed that running wheel exercises increased hippocampal neurogenesis (Kitamura et al., 2003). Mice at postnatal 3 weeks were kept for 64 days in the cage with a running wheel, and after conditioning the brain was removed and analyzed. As the result, the running wheel exercise for 64 days did not increase the volumes of the dentate gyrus (Fig. 1B).