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Population dynamics of neural progenitor cells during aging in the cerebral cortex

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ABSTRACT

Recent studies indicate that adult neurogenesis occurs in the cerebral cortex of rodents. Neural progenitor cells (NPCs) have been found in the adult cerebral cortex. These cells are expected to be regulated by various stimuli, including environmental enrichment, exercise, learning, and stress. However, it is unclear what stimuli can regulate cortical NPCs. In this study, we examined whether aging has an impact on population dynamics of NPCs in the murine cerebral cortex, using immunohistological staining for NPCs. The density of NPCs was kept from 5- to 12-month-old, dramatically decreased at 17-month-old, and thereafter maintained the same level until 24-month-old. Comparing the densities of NPCs in the cortical areas, such as the cingulate, primary motor, primary somatosensory, and insular cortices, we found that the degrees of decreased densities of NPCs in the cingulate and insular cortices were significantly smaller than those in the primary motor and somatosensory cortices. NPCs in aged cortex produced new neurons by ischemia. These results indicate that in aged mice, NPCs exist and produce new neurons in the cerebral cortex. Additionally, the extent of reduction of the density of NPCs in the cortices with higher cognitive functions may be less than that in the primary motor and somatosensory cortices.

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1. Introduction

It has been established that new neurons are generated from neural progenitor cells (NPCs) and stem cells (NSCs) and contribute to neural plasticity throughout life [1]. NSCs can produce NPCs and glial progenitor cells, and NPCs and glial progenitor cells provide new differentiated neurons and central glial cells, such as astrocytes and oligodendrocytes, respectively. Currently, NPCs and NSCs are found in some regions of the adult mammalian brains, such as the hippocampal dentate gyrus, subventricular zone, and cerebral cortex [1,2].

In the subventricular zone and the dentate gyrus, NSCs and NPCs show self-renewal and continue to produce new neurons even under healthy conditions [3,4]. Besides, newborn neurons in the olfactory bulb and the hippocampus are needed for olfactory memory [5,6], and contextual and spatial memory [7–9], respectively. These findings suggest that new neurons play an important role in the structural and functional plasticity of the adult brain.

The cerebral cortex is involved in several functions of the body and mind, including determination, intelligence, personality, planning, and motor control [10–12]. Interestingly, several studies suggest that adult neurogenesis occurs in the cerebral cortex of adult mammals [11–18]. Although cortical adult neurogenesis under healthy conditions remains controversial [19], new neurons have been reported to be induced by pathological stimuli, such as ischemia and lesion [12,14,16–18]. In addition, NPCs, which can produce new neurons in the cerebral cortex, have been first identified in the layer 1 of the cerebral cortex, and can generate inhibitory interneurons by ischemic stimuli. The cells are designated as layer 1 inhibitory neuron progenitor cells (L1-INP cells) [2,18]. Recently, chronic treatments with antidepressants have been reported to generate new neurons from L1-INP cells [20]. However, it is not clear whether other factors can induce adult neurogenesis of L1-INP cells.

Hippocampal adult neurogenesis is governed by various stimuli, including learning, exercise, environmental enrichment, and stress [21]. Among them, aging has been reported to be associated with a substantial decline of adult neurogenesis in hippocampus [22]. In this study, we examined whether the density of L1-INP cells are decreased during aging in the cerebral cortex, using

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immunostaining. We also investigated the capability of L1-INP cells for neuron production in the aged cortex.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice at 5, 12, 17, and 24 months of age (Charles River Laboratories Japan, Yokohama, Japan; 4 mice each age) were utilized. For infection with retrovirus vectors, we used male C57BL/6J mice at 5 and 24 months of age (n = 16, 20, respectively). Mice were housed (4 mice per cage) in a room with a 12 h light/dark cycle (lights on at 8:00 a.m.) with access to food and water ad libitum. All of the animal experiments were approved by the Animal Care and Use Committee of Mukogawa Women's University, based on Mukogawa Women's University's guidelines for the ethical treatment of laboratory animal (FSN-03-2015-01-A), and conducted in accordance with the principles of the Declaration of Helsinki.

2.2. Retrovirus-mediated GFP labeling of newly generated neurons

The retrovirus vector construct, pDON-5 Esyn-GFP-WPRE, was used in this study [18]. The retrovirus vectors were produced according to the manufacturer's instructions accompanying the retrovirus packaging kit (Takara Bio, Otsu, Japan). The resulting viral particles in the culture supernatant were adjusted to 1.0×10^6 transducing units/ml.

The virus injection was performed as described previously [18]. Briefly, the virus solution (0.2 μl per site) was stereotaxically injected by air pressure through a glass micropipette attached to Picospritzer III (Parker, Cleveland, OH) into the primary motor cortex (0.8−1.8 mm anterior to bregma, 1-2 mm lateral, and 0.2 mm depth below the cortical surface) [23]. One hemisphere received a total of 5 μl of the virus solution (25 injection sites in 1.0 mm × 1.0 mm square).

2.3. Ischemia treatment

Ischemia was induced according to the previous reports [18,24]. Briefly, 2 days after the virus injection, both common carotid arteries (CCAs) were transiently occluded with clamps for 10 min. Control animals were treated identically, except for occlusion of CCAs. The mice were allowed to survive for 4 weeks after ischemia.

2.4. Immunostaining

Fixation and immunofluorescence staining were performed as previously described [20]. Briefly, mice were deeply anesthetized with chloral hydrate (245 mg/kg, intraperitoneally) and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). The brain samples were mounted in Tissue-Tek (Miles, Elkhart, IN), frozen, and cut coronally into 50-μm-thick coronal sections, using a sliding microtome (model LS-113; Yamato-Kouki Co.; Saitama, Japan). After washing in PBS for 1 h, sections were incubated at 4 °C for 48 h or at room temperature overnight with the indicated primary antibodies.

For fluorescent staining, after washing in PBS for 1 h, the sections were incubated at room temperature for 1 h with secondary antibodies conjugated with fluorescent dyes. After washing in PBS, sections were mounted on glass slides coated with 3-aminopropyltriethoxysilane and embedded with Permafluor (Thermo Scientific, Pittsburgh, PA). Confocal laser-scanning microscopy (A1+, Nikon, Tokyo, Japan) was used to obtain images of the stained sections.

For immunostaining with ABC method, the sections were incubated with a biotinylated secondary antibody at room temperature for 24 h. The immunoreacti**v**e sites were **v**isualized with the avidine-biotin complex peroxidase method using the avidin-biotin complex kit (ABC kit: Vector Laboratories). A 3,3′-diamino benzidine tetrahydrochloride, 4HC1 solution containing 0.3% nickel ammonium sulfate in 0.05 M Tris-HCl, pH 7.6, was used as the substrate for peroxidase.

2.5. Antibodies

The following primary antibodies were used: mouse monoclonal antibody for Ki67 (Antibody Registry ID, AB393778; 1:20, BD Biosciences, San Jose, CA); rabbit polyclonal antibodies for GABA (Antibody Registry ID, AB477652; 1:1000, Sigma-Aldrich, St. Louis, MO) and GFP (Antibody Registry ID, AB10073917; 1:1000, Thermo Fisher Scientific). The following secondary antibodies were used: goat anti-mouse IgG Alexa Fluor 594 (1:200, Life Technologies, Carlsbad, CA), goat anti-rabbit IgG Alexa Fluor 488 (1:200, Life Technologies), goat biotinylated anti-rabbit IgG (1:200, Vector Laboratories).

2.6. Image analysis

Positive cell counting was carried out in the images obtained. Area sizes of cortical layer 1 in all captured images were measured with ImageJ. For the quantification of the numbers of immunopositive cells, we used ImageJ with WCIF ImageJ bundle (http://www.uhnres.utoronto.ca/facilities/wcif/). There are many GABA+ structures in the tissue images, such as GABA+ cells and GABA+ neurites. GABA+ neurites were excluded in cell counting. In addition, tissue images were converted into 8-bit black and white images. Image thresholds were automatically determined by a plugin “maximum entropy threshold”, and the binary images were obtained. Once the images were segmented, GABA+ cells were visualized. Round or orbital cell bodies were designated as GABA+ cells, while thin-long structures were designated as neurites. GABA+ cells were first determined, and Ki67+/GABA+ cells were identified. Densities of L1-INP cells are given as cell numbers/mm^3 (4 mice at each time point; means ± S.E.M).

To produce a distribution map of L1-INP cells, all labeled L1-INP cells, which are contained in 6 serial sections (total 300 μm), were piled up to a single plane of brain atlas (see Fig. 2A).

For quantification of new neurons, which were stained by 3,3′-diamino benzidine tetrahydrochloride, 4HCl (DAB) and nickel ammonium sulfate, all GFP-immunostained new neurons were counted in all serial sections of the virus vector-infected regions.

2.7. Statistical analysis

Changes in densities of L1-INP cells during aging of the cerebral cortex were investigated using one-way ANOVA. Differences between cortical areas and time points were compared using two-way ANOVA. Decreased density of L1-INP cells in the cingulate and insular cortices and the primary motor and somatosensory cortices was compared by *t*-test. Significant main effects and/or interactions were followed by Tukey's post hoc analyses to examine group or time differences. Adult neurogenesis of L1-INP cells in interaction of two factors, aging and ischemia, was compared by two-way ANOVA. Values are given as means ± S.E.M. GraphPad Prism (version 6, GraphPad Software, La Jolla, CA) was used to analyze all data. For all statistical analyses used, the alpha level was set at $P < 0.05$.

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