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Multiphoton microscope imaging: The behavior of neural progenitor cells in the rostral migratory stream

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Abstract

Neural progenitor cells (NPCs) in the subventricular zone (SVZ) travel a long distance along the rostral migratory stream (RMS) to give rise to interneurons in the olfactory bulb (OB). Using the multiphoton microscope and time-lapse recording techniques we here report the behavior of NPCs in the RMS under both intact and ischemic conditions in living brain slices. The NPCs were visualized in 3-week-old transgenic mice that carry the reporter gene, green fluorescent protein (GFP), driven by the nestin promoter. Cortical brain ischemia was induced by permanent occlusion of the right common carotid artery and the middle cerebral artery. We observed that the RMS contained two populations of NPCs: nonmigrating cells (bridge cells) and migrating cells. Bridge cells enabled migrating cells to travel and also produced new cells in the RMS. The direction of NPC migration in the RMS was bidirectional in both intact and ischemic conditions. Cortical ischemia impeded NPC travel in the RMS next to the lesion area during the early period of ischemia. Cell–cell contact was a prominent feature affecting NPC translocation and migratory direction. These data suggest that behavior and function of nestin-positive NPCs in the RMS are variable. Cell–cell contacts and microenvironmental changes influence NPC behavior in the RMS. This study may provide insights to help in understanding NPC biology.
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Keywords: Nestin; Neural progenitor cell; RMS; Cell behavior; Brain ischemia; Chain migration

Neural stem cells/neural progenitor cells (NSCs/NPCs) with the capacity of self-renewal and multiple linage differentiation have been well documented to reside in the subgranular zone (SGZ) in the dentate gyrus of the hippocampus and the subventricular zone (SVZ) in the forebrain throughout mammalian life span [8]. The NSCs/NPCs in the SGZ give rise to granular cells locally, whereas the NSCs/NPCs in the SVZ travel a relatively long distance (about 5 mm in adult mice) [14], along the RMS to the OB where they differentiate into local interneurons [8,13,14,16]. Convincing evidence has shown that migrating neuroblasts in the RMS form chains [5,15], allowing NSCs/NPCs to touch and use each other as migratory substrates moving forward to their destination, the OB [4,15].

Nestin, an intermediate filament, was first characterized in neuroepithelial stem cells during embryogenesis in rats [12]. Nestin, used as a marker for neural progenitor cells in the brain, was expressed in the SVZ and in the migrating neuroblasts in the RMS [1,6,22,26].

NSC/NPC proliferation and differentiation in the SVZ is triggered by focal brain ischemia [2,10,21]. NSCs/NPCs migrate to the infarct areas [2,11], suggesting NSCs/NPCs play a role in brain self-repair after brain ischemia [2,24].

A multiphoton microscope equipped with two low-energy photon sources has very limited phototoxicity. Therefore, multiphoton microscopy is a key technology that allows us to observe cell behavior and cellular biological activity in live animals or living brain slices.

Although numerous previous studies have investigated neuroblasts migration in the RMS using fixed brain tissue, there

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is little knowledge based on live imaging of NSCs/NPCs in response to brain ischemia. In the present study we took advantage of the transgenic mice expressing GFP driven by nestin promoter (nestin-GFP) and multiphoton microscopy to observe normal behavior of nestin-GFP cells in intact brain and to see how NSCs/NPCs in the RMS respond to cortical brain ischemia in living brain slices.

Experimental protocol for this study was approved by the Institutional Animal Care and Use Committee. In nestin-GFP mice, GFP, a reporter gene, was driven by the second intronic enhancer of the nestin gene and the thymidine kinase minimal promoter. Nestin-GFP mice were generated in the transgenic mouse cores at Children's Hospital Boston and Brigham and Women's Hospital. Nestin-GFP positive cells in the transgenic mice are located in the ependymal wall, SVZ, and RMS in neonatal and adult transgenic mice, and share the features of NPCs and neuroblasts (Walker et al., unpublished observation). Since the size of the RMS was wider and the nestin-GFP signal was much brighter in young mice (postnatal day less than 4 weeks) than adult mice, we used 3-week-old mice for this study.

Cortical brain ischemia was produced by permanent ligation of the right common carotid artery and the middle cerebral artery in male nestin-GFP mice. Three hours after brain ischemia, mice were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), the brains were quickly removed and sagittal brain slices (300 μm thick) were cut on ice with a vibratome. Brain slices with the RMS and SVZ were selected, and placed in a chamber containing artificial cerebral spinal fluid (maintained at 37 °C) with 95% O_2 and 5% CO_2 during time-lapse recoding under a multiphoton microscope. Using a 40× objective (Olympus 40× water/infrared lens, NA 0.8), nestin-GFP imaging in the RMS and/or SVZ of both intact and ischemic brains was acquired at 7-min intervals over a period of 15 h. To determine cell migratory speed, 3–5 cells ($n = 3$–4 slices) that remained in the plane of focus during recording were selected. Images were obtained from the surface of the slice to 50–60 μm deep.

Multiphoton microscopy was performed using an ultrafast Ti: sapphire laser (Mira 900, Coherent, Santa Clara, CA) interfaced with an upright Olympus confocal microscope (BX61W) and scanhead (FluoView). The laser excitation wave was set at 760–890 nm. Emission was collected in the scanhead using two multialkali photomultiplier tubes set at optimal voltage-gain-offset. Images were collected as 512 × 512 pixels × 1 μm × 8-bit files (50–60 sections/stack) using Fluoview software. Time-resolved fluorescence was measured by capturing continuous images as fast as 0.1 s. Image analysis was performed using Zeiss LSM 510 software and figures were constructed using Image J software. Three-dimensional reconstruction of the nestin-GFP cells in the RMS from *z*-series stacks was used for analyzing NPC's behavioral.

Nestin-GFP cells in the RMS appeared as chains or clusters. In the present study, for the first time, we classified nestin-GFP cells in the RMS as bridge cells or migrating cells based on cell mobility ($n = 204$ cells). In intact mice, it was obvious that some of the nestin-GFP cells in the RMS moved (∼44% of the cells). These cells are the traveling or migrating cells (Fig. 1B and C, and supplementary Movie 1). In contrast, some of the nestin-GFP cells in the RMS did not move, stayed in the same position, or slightly moved around locally over time (more than 2–3 h, ∼56% cells), serving as a bridge for cell travel (Fig. 1D and supplementary Movie 1). Therefore, these cells are named bridge cells. Multiple directions of movement of nestin-GFP cells were observed in the RMS. Some of the cells moved forward in the direction of the OB (∼25% cells, Fig. 1B and supplementary Movie 1) whereas other cells moved in the opposite direction, migrating forward in the direction of the SVZ (∼6% cells, Fig. 1C and supplementary Movie 1). In addition, some of cells moved orthogonally in the RMS (∼13% cells). In the time-lapse recording, some of nestin-GFP cells (∼4% cells) in the RMS were still dividing (supplementary Fig. 4 and Movie 1). Some nestin-GFP cells moved around each other, either approaching or maintaining a certain distance from one another (Fig. 1D and supplementary Movie 1). Bridge cells could suddenly change their position to facilitate movement of migrating cells (supplementary Fig. 5 and Movie 1).

Bridge cells and migrating cells in the RMS responded to brain ischemia differently. The location of infarct core in the cortex and recording area was shown in supplementary Fig. 6. During the first 5 h period of recording (3–8 h post-ischemia), no traveling cells were observed but only bridge cells remained in the RMS next to the area beyond ischemic zone (Fig. 2, and supplementary Movie 2). In the RMS distal to the area of infarct, there were traveling cells that made transient contact with migrating cells or bridge cells. After such contacts, the migratory direction was changed (Fig. 2C, 3–12 and supplementary Movie 2). Interestingly, when a traveling cell touched a bridge cell on its way towards the SVZ, it reversed the cell migratory direction (Fig. 2C, 13–17 and supplementary Movie 2). During the second 5 h period (8–13 h post-ischemia), cell behavior similar to that in the first 5 h recoding period was observed (supplementary Movie 3). During the last 5 h period of recording (13–18 h post-ischemia), the number of bridge cells was reduced and no clear chain of cells remained in the RMS although the rostral part of RMS retained some moving cells. Interestingly, two cells with enlarged cell bodies touched each other momentarily, and then extended long leading processes and moved toward the SVZ. When one of them touched a bridge cell, it began to reverse direction (Fig. 3B and supplementary Movie 4). These data suggest that cortical brain ischemia interrupted ongoing cell migration in the RMS. The traveling cells moved away from the ischemic area, bridge cells were more tolerant to ischemia during the very early phase of ischemia, and cell–cell contact influences cell behavior.

In intact mice, migration speed for nestin-GFP cells was 30 ± 1.6 μm/h (mean ± S.E.). During 3–8 h after ischemia, cell migration speed was markedly reduced (20.5 ± 0.3 μm/h). However, it recovered during the period of 13–18 h post-ischemia (30.7 ± 1.2 μm/h). The reduction of migration speed in the early ischemia was significantly different when compared to intact and 13–18 h post-ischema ($p < 0.01$, one-way ANOVA test followed by a post hoc Bonferroni correction).

Overall, we observed that: (1) there were two populations of cells expressing nestin in the RMS: nonmigrating cells (bridge cells) and migrating cells; (2) cells migrated in mul-

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