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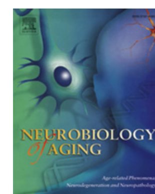

Age-dependent regional changes in the rostral migratory stream

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A B S T R A C T

Throughout life the subventricular zone (SVZ) is a source of new olfactory bulb (OB) interneurons. From the SVZ, neuroblasts migrate tangentially through the rostral migratory stream (RMS), a restricted route approximately 5 mm long in mice, reaching the OB within 10–14 days. Within the OB, neuroblasts migrate radially to the granule and glomerular layers where they differentiate into granule and periglomerular (PG) cells and integrate into existing synaptic circuits. SVZ neurogenesis decreases with age, and might be a factor in age-related olfactory deficits. However, the effect of aging on the RMS and on the differentiation of interneuron subpopulations remains poorly understood. Here, we examine RMS cytoarchitecture, neuroblast proliferation and clearance from the RMS, and PG cell subpopulations at 6, 12, 18, and 23 months of age. We find that aging affects the area occupied by newly generated cells within the RMS and regional proliferation, and the clearance of neuroblasts from the RMS and PG cell subpopulations and distribution remain stable.

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1. Introduction

Neurogenesis occurs in the mouse subventricular zone (SVZ) throughout life and is a source of new interneurons in the olfactory bulb (OB) (Doetsch et al., 1997; Lledo and Saghatelyan, 2005; Lledo et al., 2008; Whitman and Greer, 2009). Neuroblasts migrate tangentially to the OB via the rostral migratory stream (RMS), a restricted route that is approximately 5 mm in length in mice (Lois and Alvarez-Buylla, 1994; Whitman et al., 2009). During migration, neuroblasts retain the ability to divide but have a longer cell cycle than proliferative cells in the SVZ (17.3 vs. 12.5–14 hours), and maintain an average migration rate of 23 μm/h (Luskin and Boone, 1994; Menezes et al., 1995; Morshead and van der Kooy, 1992; Poon et al., 2010; Smith and Luskin, 1998).

The outer border of the RMS is defined by astrocytes that give the RMS the appearance of a tube-like structure (Lois et al., 1996; Whitman et al., 2009). The vasculature is pervasive within the astrocytic tube, and longitudinally oriented in parallel with the migrating neuroblasts (Whitman et al., 2009). Together, the RMS vasculature and astrocytic scaffolding form a permissive environment for neuroblast migration (Bozoyan et al., 2012; García-Marqués et al., 2010; Whitman and Greer, 2009; Whitman et al., 2009). The RMS can be divided into 3 distinct anatomical regions (Fig. 1A): (1) The vertical arm (VA) extends from the SVZ and includes the descending region underlying the white matter of the corpus callosum; (2) the elbow is composed of the rostral curve toward the OB at the base of the VA; and (3) the horizontal arm (HA) is the final rostral extension into the OB (De Marchis et al., 2004).

Neuroblasts from the SVZ reach the OB 10–14 days after cell division, where they migrate radially to the OB granule and glomerular layers (GLs), differentiate, and become integrated into synaptic circuits (Carleton et al., 2003; Kelsch et al., 2010; Whitman and Greer, 2007b). Activity-dependent processes can modulate survival of newly arrived cells, but under normal housing conditions approximately 50% are integrated into the OB circuitry and the remaining are lost (Lemasson et al., 2005; Mandairon et al., 2003; Mouret et al., 2008; Sultan et al., 2011). Most of the surviving neurons differentiate into granule cells, although approximately 5% become periglomerular (PG) cells (Lemasson et al., 2005).

With only a few exceptions, the most numerous interneurons found in the OB, granule cells, are a molecularly homogeneous

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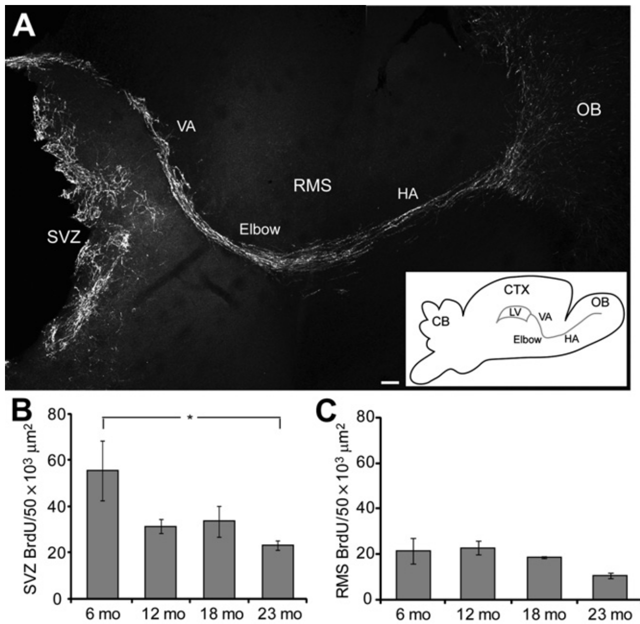

Fig. 1. Cell proliferation decreases with age in the SVZ and RMS. (A) The SVZ and RMS were delineated by DCX labeling to the beginning of the OB. Scale bar = 100 µm. Inset, diagram of the brain, LV and regions of the RMS. (B) and (C) BrdU-positive cell density in the SVZ and RMS 2 hours after BrdU injection ($n = 4$ per age group). (B) SVZ, 1-way ANOVA, $F(3,30) = 3.855$, $p < 0.05$; Bonferroni post hoc tests, 6 versus 23 months, $p < 0.05$, indicated by asterisk. (C) RMS showed a decreasing trend; 1-way ANOVA, $p > 0.05$. Abbreviations: ANOVA, analysis of variance; BrdU, 5-bromo-2′-deoxyuridine; CB, cerebellum; CTX, cortex; DCX, doublecortin; HA, horizontal arm; LV, lateral ventricle; OB, olfactory bulb; RMS, rostral migratory stream; SVZ, subventricular zone; VA, vertical arm.

population (Imamura et al., 2006; Stenman et al., 2003). However, PG cells, which surround the OB glomeruli, are a heterogeneous population of interneurons. PG cells are categorized molecularly by neurotransmitter type (dopaminergic or GABAergic) and the expression of calcium binding proteins including parvalbumin, calbindin, and calretinin (Kosaka et al., 1995, 1998; Whitman and Greer, 2007a). PG cells receive synapses from olfactory sensory neuron axons, establish reciprocal dendrodendritic synapses with mitral and tufted cells, and send axons to neighboring glomeruli (Aungst et al., 2003; Kasowski et al., 1999; Kiyokage et al., 2010). PG cells thus modulate both intra- and interglomerular circuits and affect patterns of odorant-induced activity (Gire and Schoppa, 2009; Schoppa and Urban, 2003).

With age, deficits have been reported in olfactory discrimination and sensitivity that might reflect a decline in SVZ neurogenesis (Doty et al., 2011; Enwere et al., 2004; Mandairon et al., 2011a; Patel and Larson, 2009; Rey et al., 2012; Tropepe et al., 1997). Experimentally induced reduction of adult neurogenesis results in a loss of innate olfactory responses and a diminished ability to learn olfactory-related tasks (Moreno et al., 2009; Sakamoto et al., 2011; Valley et al., 2009). Age-related decreases in SVZ neurogenesis might be caused in part by a decrease in the number of dividing cells and diminished expression of growth factors or their signaling pathways (Enwere et al., 2004; Jin et al., 2003; Maslov et al., 2004; Shook et al., 2012; Tropepe et al., 1997).

Here, we assessed age-dependent changes in the organization of the RMS and the effects of age-related decreases in neurogenesis on subpopulations of PG cells. Cell division continues within the RMS but is affected by age in a regional manner. We have established that neuroblast migration capability is unaffected by age, although there is a decrease in the area of the RMS occupied by migrating neuroblasts resulting from a decrease in neurogenesis. Despite the age-related decline in neurogenesis, the number and distribution of PG cells remains stable.

2. Methods

2.1. Animals and 5-bromo-2′-deoxyuridine administration

C57 mice (National Institute on Aging, Charles River Laboratories) from each age group (6 months, $n = 8$; 12 months, $n = 8$; 18 months, $n = 8$; 23 months, $n = 8$) received 2 intraperitoneal injections of 5-bromo-2′-deoxyuridine (BrdU, 50 mg/kg) 2 hours apart. Mice were euthanized 2 hours or 14 days (for the RMS study) or 28 days (for the PG cell study) after BrdU injection. Mice were anesthetized with pentobarbital and perfused transcardially with 0.1 M phosphate buffered saline (PBS) with 1 unit/mL heparin, followed by 4% paraformaldehyde (PFA). Brains were postfixed in 4% PFA for 2 hours after dissection, rinsed in PBS overnight, cryoprotected in 30% sucrose in PBS, transferred to OCT (Optimum Cutting Temperature, Tissue-Tek) and rapidly frozen in a slurry of dry ice and ethanol and stored at −80 °C until used.

2.2. Immunohistochemistry

Brains were sectioned on a cryostat into 20-µm coronal sections and mounted on Superfrost Plus slides, or 50-µm free-floating sagittal sections were collected in 48-well tissue culture plates (Becton Dickinson Labware) in antifreeze solution (30% sucrose, 30% ethylene glycol, 1% polyvinyl pirrolidone in 0.1 M phosphate buffer) and stored at −20 °C. Slides of coronal sections were dried in a 37 °C oven for 15 minutes. All sections were incubated in 0.025 M HCl at 65 °C for 30 minutes, 0.1 M borate buffer for 10 minutes, and then PBS for 5 minutes. For PG cell immunostaining, sections were incubated in Tris buffered saline (TBS)—0.3% Triton X-100 (TBS-T) for 15 minutes and blocked for 1 hour in TBS-T with 3% bovine serum albumin (BSA) and 5% normal donkey serum (cat. #017-000-121; Jackson ImmunoResearch, West Grove, PA, USA). For RMS immunostaining, free-floating sections were blocked for 30 minutes in 3% BSA PBS-Triton X-100 (PBS-T).

Incubation with primary antibody in PBS-T (free floating) or TBS-T (slides) with 3% BSA and 5% normal donkey serum was overnight at 4 °C with the following primary antibodies: mouse monoclonal anti-BrdU (1:200; cat. #346580, Becton Dickinson, San Jose, CA, USA); rabbit polyclonal anti-Ki67 (1:1000; cat. # NB110-89719SS, Novus Biologicals, Littleton, CO, USA); rabbit polyclonal anti-tyrosine hydroxylase (1:2000; cat. #AB152, Millipore, Temecula, CA, USA); rabbit polyclonal anti-calbindin D28K (1:1000; cat. #AB1778, Millipore); mouse monoclonal anti-calretinin (1:800; cat. #MAB1568, Millipore); mouse monoclonal anti-neuronal nuclei (NeuN; 1:700; cat. #MAB377, Millipore); goat anti-doublecortin (DCX; 1:500; cat. #sc-8066, Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA); rat anti-platelet endothelial cell adhesion molecule (PECAM; 1:50; cat. # 553370, BD Pharmingen, San Diego, CA, USA); rabbit anti-glial fibrillary acidic protein (GFAP; 1:1000; cat. # M0761, DAKO, Carpinteria, CA, USA); rabbit anti-cleaved caspase-3 (1:100; cat. #9661, Cell Signaling Technology, Danvers, MA, USA). Secondary antibodies were applied for 1 hour at room temperature in 3% BSA PBS-T or TBS-T (cat. #'s A21121, A21124, A21240, A31573, A31573; 1:1000; Invitrogen, Eugene, OR, USA) with the nuclear marker DRAQ5 (1:1000; cat. # DR71000, Biostatus Limited, Leicestershire, UK) or DAPI (diamidino-2-phenylindole; 1:500; cat. #D1306, Invitrogen). For double-labeling with 2 mouse primary antibodies, slides were fixed with 2% PFA in PBS for 15 minutes after the first primary and secondary antibody staining. The second primary and secondary antibodies were then applied as described. Lipofuscin fluorescence was reduced by staining with 1% Sudan Black in 70%

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