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Magnetic resonance imaging of odorant activity-dependent migration of neural precursor cells and olfactory bulb growth

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ABSTRACT

Neural progenitors or neuroblasts are produced by precursor cells in the subventricular zone (SVZ) and migrate along the rostral migratory stream (RMS) to the olfactory bulbs (OB) throughout life. In the OB, these adult born neurons either die or replace existing olfactory interneurons, playing a critical role in the stabilization of OB circuitry. Although several aspects of the addition of new neurons into the OB have been studied, it is unclear whether long-distance activity from the OB can regulate the influx of migrating neuroblasts along the RMS. In this study, iron oxide-assisted MRI was used to track the migration of neuroblasts in combination with reversible naris occlusion to manipulate odorant-induced activity. It was found that decreasing olfactory activity led to a decrease in the rate of neuroblast migration along the RMS. Removal of the naris occlusion led to an increase in migratory rate back to control levels, indicating that olfactory activity has regulatory function on neuroblast migration in the RMS. Blocking odorant activity also led to an arrest in OB growth and re-opening the block led to a rapid re-growth returning the bulb size to control levels. Furthermore, pharmacogenetic elimination of the neuroblasts demonstrated that they were required for re-growth of the bulb following sensory deprivation. Together, these results show that sensory activity, neural migration and OB growth are tightly coupled in an interdependent manner.

1. Introduction

The olfactory bulb (OB) is the first brain area required for processing odor information. Odorants bind to receptors on olfactory sensory neurons in the nasal epithelium and project their axons to the glomerular layer of the OB. Mitral cells relay sensory inputs from the glomerular layer to the piriform cortex in the brain. Throughout life, the OB displays remarkable plasticity including regeneration of olfactory neurons and integration of adult born neurons by ongoing neurogenesis in the subventricular zone (SVZ) lining the lateral ventricle wall of the forebrain (Lois and Alvarez-Buylla, 1994). In the SVZ, neural stem cells give rise to neural precursor cells called neuroblasts, which organize themselves into chains as they migrate along the rostal migratory stream (RMS) (Lois and Alvarez-Buylla, 1994; Lois et al., 1996). These neuroblasts migrate tangentially into the OBs (Doetsch and Alvarez-Buylla, 1996; Lois et al., 1996; Peretto et al., 1997) and then migrate radially to their specific destinations within the different layers of the OB, primarily the glomerular layer (GL) and granule cell layer (GCL) (Batista-Brito et al., 2008). These new neurons integrate into microcircuits and participate in odor processing (Livneh et al., 2014). In addition, these new neurons are critical for proper formation and maintenance of the intrabulbar connectivity map (Cummings and Belluscio, 2008; 2010).

Olfactory activity has been identified as a key regulator of survival of neural progenitors, their final cell type distribution, and their turnover in the olfactory bulb (Yamaguchi and Mori, 2005). However, little is known about the effects of olfactory activity on the dynamics of cell migration along the SVZ-RMS-OB pathway. While dynamics of neuroblast migration have been elucidated in acute slice cultures (Luskin and Boone, 1994; Koizumi et al., 2006; Nam et al., 2007; Martinez-Molina et al., 2011; Khlghatyan and Saghatelyan, 2012), assessing the effects of olfactory input on RMS dynamics *in vivo* has been challenging due to a lack of tools that can monitor the long migratory path and that allow for

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longitudinal experimentation in live animals. Two-photon imaging techniques are useful for studying of the superficial areas in the OB such as the glomerular layer (Sawada et al., 2011; Liang et al., 2016). However, these tools cannot image long-distance migration of the cells from the SVZ to the olfactory bulb *in vivo*.

In this study, we utilized neuroblast labeling with micron-sized iron oxide particles (MPIOs), which has enabled *in vivo* MRI imaging of cell migration along the RMS into the OB (Shapiro et al., 2006a,b, Sumner et al., 2009; Granot et al., 2011), in combination with a reversible naris occlusion model (Cummings et al., 1997) to investigate the effects of odorant-induced activity on growth of the OB and migration dynamics of new neurons. Blockade of olfactory activity in three-week-old rats by naris occlusion led to a cessation in growth of the affected OB and a significant decrease in the migration rates of neuroblasts along the RMS. Removal of the naris occlusion to restore normal olfactory sensory stimulation led to an increase in growth of the OB and an increase in migration rate. Furthermore, the importance of ongoing neurogenesis for the recovery of olfactory bulb size after removal of the naris occlusion was tested in a transgenic rat model whose neuroblasts could be pharmacogenetically ablated and showed that ongoing neurogenesis is indeed required for the re-growth of the olfactory bulb following the reinstatement of normal levels of olfactory activity. These results demonstrate the usefulness of combining MRI cell tracking with MRI anatomical measurement to elucidate a tight coupling of olfactory activity, neuroblast migration and maturation of the rodent olfactory bulb.

2. Materials and methods

2.1. Animal procedures

All animal procedures were done according to the guidelines of Institute of Laboratory Research Council and approved by the Animal Care and Use Committee (ACUC) of the National Institute of Neurological Disorders and Stroke at the National Institutes of Health.

2.2. Unilateral naris occlusion

To deprive animals of olfactory sensory input, 3-week-old, male, Sprague-Dawley rats were subjected to reversible unilateral naris occlusion (Cummings et al., 1997; Marks et al., 2006). Polyethylene tubing of various diameters was used to construct nose plugs, which were adjusted to fit the nostrils of the animals. The nose plugs were replaced every 5–6 days to keep up with the increasing size of the nostrils as the animals grew. MRI images of the OB were performed weekly following the occlusion to obtain a dynamic measurement of the change of OB volume. All MRI experiments were performed on an 11.7 T animal MRI system (30 cm 11.7 T horizontal magnet, Magnex Scientific, Oxford, England, MRI Electronics, Bruker Biospin, Billerica, MA), equipped with a 12-cm integrated gradient shim system (Resonance Research Inc, Billerica, MA). A custom-built volume transmit coil and a custom built, 2.5-cm-diameter, receive-only surface-coil were used for MRI. 3D gradient echo sequences were used for all MRI acquisitions. The following parameters were used: Field of View (FOV) = 1.92 cm × 1.92 cm x 1.92 cm, matrix size 256 × 256 × 256 (75-μm nominal isotropic resolution), 12.5 kHz bandwidth, echo time (TE) = 8 ms, repetition time (TR) = 25 ms, and flip angle = 8°. OB volumes were obtained from manually drawn serial voxel of interest (VOI) that covered the entire OB using the Medical Image Processing, Analysis, and Visualization (MIPAV) program (http://mipav.cit.nih.gov) (Saar et al., 2015).

2.3. In situ MRI cell labeling with micron-sized iron oxide particles (MPIOs)

For *in situ* cell labeling, in one group of the animals 20-μL of MPIOs (average diameter of 1.63 μm, Bangs Laboratories, used as received) suspension were injected after 3 weeks of occlusion (6-weeks of age) into the lateral ventricle near SVZ (coordinate: AP+1.9 to +2.0, ML +1.9 to +2.0, DV -4.0 from bregma) ipsilateral to the side of the occluded naris. In the other two groups of animals, the naris plug was removed after 3 weeks of occlusion, to restore olfactory activity. MPIOs were injected 2 or 7 days prior to removal of the naris plug. MPIO injection allows a portion of the migrating neuroblasts to be labeled near the SVZ and then carry the MPIOs as they migrate into the OB. Serial *in vivo* MRI was performed to monitor the appearance of hypointense signal that was generated from MPIOs using the following parameter: Field of View (FOV) = 1.92 cm × 1.92 cm ×1.92 cm, matrix size 256 × 256 x 256 (75-μm isotropic resolution), 12.5 kHz bandwidth, echo time (TE) = 8 ms, repetition time (TR) = 25 ms, and flip angle = 8°. For detection of the migrating stream of neuroblasts, MRI was performed at 4–6 h post-injection and then at 20–24 h post-injection. The rate of cell migration was calculated as the distance the migrating neurons traveled in the time between the two imaging sessions. The number of hypo-intense voxels within the OB was counted based on voxels that had 30% or more decrease in signal intensity. Three 75-μm thick MRI slices were combined through minimum intensity projections to increase counting efficiency minimizing the chance that repetitive counting of the same voxels occurred. It was assumed that each voxel with decreased signal intensity contained a single cell. The density of MPIO-containing cells was derived by dividing the number of spots by the OB volume.

2.4. Pharmacogenetic ablation of neurogenesis in GFAP-TK transgenic rats and valganciclovir (VGCV) administration

Transgenic rats that express herpes simplex virus thymidine kinase under the glial fibrillary acidic protein (GFAP) promoter (GFAP-TK), on a Long-Evans background were used to elucidate the effect of neurogenesis on OB volume. VGCV treatment of GFAP-TK rats inhibits adult neurogenesis by selectively killing GFAP-expressing proliferating cells in the SV zone, as described previously (Snyder et al., 2016). Unilateral naris occlusion was introduced on the same day of the VGCV treatment on both transgenic (TK) and wild-type rats (WT). After 3 weeks of occlusion and VGCV treatment, the animals were sacrificed and perfused with 4% parafomaldehyle (PFA) in PBS containing 5 mM of gadolium-DTPA (Magnevist). To keep the OB tissue intact and prevent distortion of the OB, the brains were left in the skulls and transferred to a 50-mL conical tube. The same tube was then filled with the fixative containing gadolinium-DTPA. *Ex vivo* MRI was performed to obtain images of the OB using the following parameters: For *ex vivo* imaging the following MRI parameters were used: FOV = 2.56 cm × 2. 56 cm × 2.56 cm, matrix size 342 × 342 × 342, 20 kHz bandwidth, TE = 10 ms, TR = 25 ms, and flip angle = 30°. One set of control animals continued the VGCV treatment but had the naris plug removed for 3 weeks before perfusion. A second set of control animals had received 3 weeks of naris occlusion but no treatment with VGVC.

2.5. Immunohistochemistry

After the MRI acquisitions, animals were perfused transcardially with 1x PBS followed by 4% PFA. The brains were post-fixed, cryoprotected, sectioned (40 μm) coronally, and processed for immunofluorescence for neuroblast and interneurons. For immunostaining of neuroblasts, anti-doublecortin (DCX) (guinea pig anti-doublecortin, Millipore, antibodiy dilution 1:400) was used. For mature interneurons in the OB, anti-calretinin (rabbit anti-calretinin; Abcam; antibody dilution 1:2500) and anti-5T4 (mouse anti-5T4: Abcam; antibody dilution 1:1000) were used. Appropriate secondary antibodies conjugated to Cy3 (Jackson ImmunoResearch) were used for counter staining. Sections were mounted serially onto slides and coverslipped using Vectashield mounting medium (Vector Laboratories). Confocal images were collected with a Zeiss LSM 510 laser scanning confocal microscope using a 40x, 0.9 NA oil objective. Fluorescence images of doublecortin in the SVZ-RMS of GFAP-TK and WT rats were images on Nikon ECLIPES Ti microscope (Nikon,