

Single Cell Tracking of Neural Progenitors labeled in vivo with Micron Sized Particles of Iron Oxide (MPIO) into Specific Layers of the Olfactory Bulb

J. P. Sumner¹, S. Dodd¹, E. Wayne^{1,2}, D.-Y. Chen¹, Y. Chen^{1,3}, D. Maric^{1,4}, and A. P. Koretsky¹

¹National Institutes of Health, Bethesda, MD, United States, ²University of Pennsylvania, Philadelphia, PA, United States, ³National Institute of Standards and Technology, ⁴NINDS FACS Facility, Bethesda, MD, United States

Introduction

Rodent neural progenitor cells (NPCs) continually populate the olfactory bulb (OB). Once in the olfactory bulb, these NPCs migrate radially and integrate into pre-existing neural circuitry. Based on histological evidence, the NPC distribution within the OB as well as their number can be effected when animals are continually exposed to odors [1,2]. However, a recent report provides contradictory evidence that neither the number nor the distribution of NPCs in the olfactory bulb changes with odor [3]. Since these studies relied exclusively on histology, it remains unclear whether odor would effect the NPC distribution globally in the olfactory bulb of a live animal. To overcome this limitation, MRI was used to characterize the distribution of NPCs in the olfactory bulb of both naïve and odor stimulated animals. It has previously been demonstrated that micron particles of iron oxide (MPIOs) can be used to label endogenous neural precursors after direct injection of MPIOs and migration of NPCs detected with MRI [4, 5]. It has also been demonstrated that Manganese enhanced MRI (MEMRI) can delineate layers present in the olfactory bulb [6]. Here MPIOs were used to label the neural progenitors that originate in the subventricular zone of adult rodents. At four weeks post injection, the animals were imaged at 50µm isotropic resolution. At this resolution individual NPCs could be identified and counted within the olfactory bulb in a layer specific manner with the aid of MEMRI.

Materials and Methods

Seven, 6 week old Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA) were stereotactically injected with 1.4×10^8 MPIOs (Bangs Laboratories, Inc., Fishers, IN). Four animals were exposed to amyl acetate for 4 weeks. Three animals were not exposed to amyl acetate (naïve). Prior to MRI all animals were infused w/ 37mg MnCl₂/kg. MRI data was acquired 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 12 cm-id gradients, Resonance Research Inc, Billerica, MA) using a volume transmit coil and a custom built, 1 cm diameter, receive-only surface-coil. 3D Multi-gradient echo (MGE) sequences were used for MRI with the following parameters: FOV 1.28 cm x 1.44 cm x 0.96 cm, Matrix 256 x 288 x 192 (50 µm isotropic resolution), 50 kHz bandwidth, multiple TEs 4.25, 11.75, 19.25, and 26.75 ms, and TR 32 ms. Images were reconstructed using IDL. Images from the second and third echo were thresholded at 3 x standard deviation of the noise of the surrounding pixels to select the MPIOs from the background. This thresholded mask was overlaid on the original images from the first echo. From these composite images, the MPIOs were counted in each layer of the olfactory bulb.

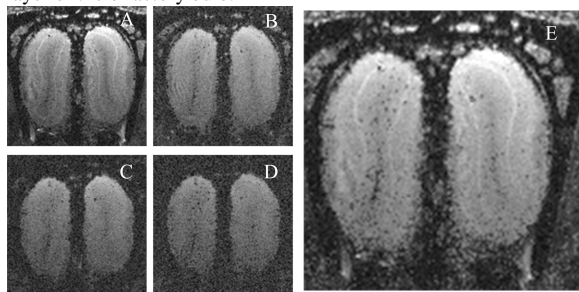


Figure 1: MRI at TE A) 4.25 ms B) 11.75 ms C) 19.25 ms D) 26.75 ms. Panel E is the thresholded image overlaid on the first echo image showing dark spots due to cell labeled MPIOs on olfactory layers.

Results

A single MRI sequence was used to capture both T₁ and T₂* weighted images at 50µm isotropic resolution as shown in Fig. 1. The olfactory bulb image acquired with the first echo is T₁ weighted and shows the Mn²⁺ enhanced layers of the bulb. The next three echoes acquired in the sequence are T₂* weighted. Hypointense spots indicate the presence of MPIOs. MPIOs could be overlaid on the T₁ weighted image to form a composite image. From this composite image, the MPIOs could be counted in a layer specific manner in the olfactory bulb. Olfactory bulbs from naïve and odor exposed animals were compared using flow cytometric analysis and MRI. The results are shown in Figure 2. The FACS analysis showed that there was no significant difference between naïve and odor exposed animals in the number of GAD67+, tyrosine hydroxylase+, and MPIO+ cells. Though the total number of cells was similar, MRI showed that the distribution within the olfactory bulb differed. There was no significant difference in MPIOs counted in the glomerular (GL) or external plexiform layer (EPL) but there was twice as many MPIO labeled NPCs in the mitral cell layer (MI) of odor exposed animals compared to naïve animals.

Discussion

MEMRI can be used with MPIOs to detect NPCs in the olfactory bulb in a layer specific manner. Cells could be detected in each layer of the olfactory bulb. While total number of cells was similar in both odor exposed and naïve animals, there were twice as many cells identified in the odor exposed animals compared to naïve animals in the mitral cell layer. In addition, a high concentration of MPIO+ cells were identified in the MI layer of the odor exposed animals in the same region where amyl acetate exposure resulted in glomeruli being activated [7]. These NPCs in the mitral cell layer may be necessary for olfaction for the purpose of modulating the sensory signal from the glomeruli. Future work will determine the specific cell fate of the MPIO labeled NPCs that enter the MI due to odor exposure. Furthermore, it will be interesting to combine functional MRI approaches with the cell labeling approaches demonstrated in this work to determine whether NPCs and functional activated regions are co-localized.

References

1. Rosselli-Austin, L., et al. Dev Brain Res, 1990, 51, 135.
2. Lledo, PM., et al. J Neurosci., 2002, 22, 2679.
3. Magavi, SSP., et al. J Neurosci., 2005, 25, 10729.
4. Shapiro, EM., et al. NeuroImage, 2006, 32, 1150.
5. Sumner, JP., et al. NeuroImage, 2008, In Press <http://dx.doi.org/10.1016/j.neuroimage.2008.07.050>
6. Aoki, I., et al. NeuroImage, 2004, 22, 1046.
7. Xu, F., et al. PNAS, 2000, 97, 10601.

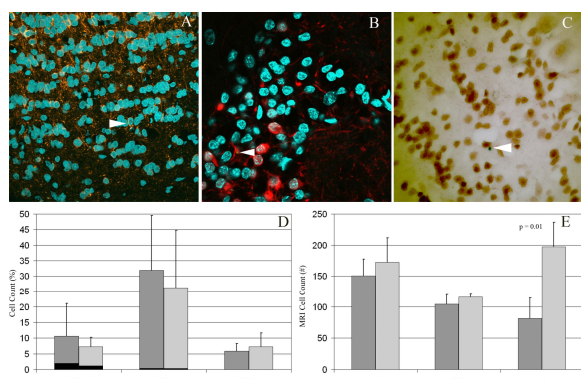


Figure 2: MPIO+ cells in the OB where panel A) GAD 67+ and B) TH+ stained cells that contain MPIOs (green). Panel C is a Prussian blue stain. Arrows indicate MPIOs. Panel D are results from FACS analysis of the OB for naïve and odor exposed animals. Naïve n=5, Odor n=4. 20% of the GAD 67 cells and 5% of the TH were MPIO+ for both odor and naïve animals (shown as black bars). Panel E are the results of cell counts from the MRI images. There were significantly more MPIOs observed in the MI layer of the odor exposed animal.