MRI Tracking of Endogenous Neural Precursors Odor Induced Accumulation in the Mitral Cell Layer of the Rodent Olfactory Bulb

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Introduction

Rodent neural progenitor cells (NPCs) continually populate the olfactory bulb (OB) and integrate into pre-excising neural circuitry. Based primarily on histological evidence, the NPCs distribution within the OB and number can be altered 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 are affected by odor enrichment [3]. Since these studies relied on histology, it remains unclear whether the limited ability to quantify the NPCs distribution throughout the olfactory bulb leads to inconsistent results. To overcome this limitation, we demonstrate that high resolution MRI can be used to characterize the distribution of neural progenitors in the olfactory bulb of both naïve and odor enriched animals. It has previously been demonstrated that micron sized particles of iron oxide (MPIOs) can be used to label endogenous neural precursors enabling MRI their migration into the olfactory bulb [4, 5]. Previously it has been demonstrated that manganese enhanced MRI (MEMRI) can detect anatomical layers in the olfactory bulb [6]. In this study MPIO labeled neural progenitors were imaged in the olfactory bulb at 50µm isotropic resolution. At this resolution individually labeled 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 $1.4x10^8$ MPIOs (Bangs Laboratories, Inc., Fishers, IN). Four animals were exposed to amyl acetate for 4 weeks. Three animals had no exposure to amyl acetate. Prior to MRI all animals were infused w/ 37mg Mn^{2+}/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, and 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 images from the first echo which enabled alignment and counting of MPIO labeled cells in each layer of the olfactory bulb.

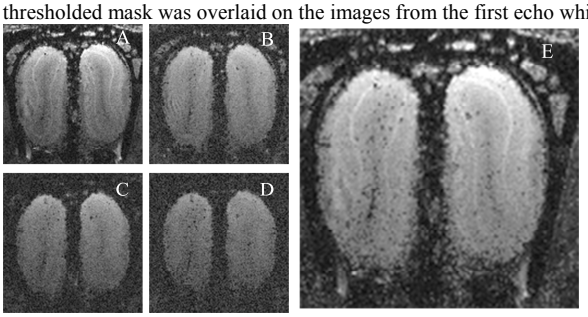

Figure 1: MRI at TE A) 4.25 ms B) 11.75 ms C) 19.25 ms D) 26.75 ms. E) Thresholded image overlaid on the first echo. Dark spots are the MPIOs and bright enhancement is due to Mn^{2+}.

Results

A multi-gradient echo sequence was used to capture both T_1 and T_2* weighted images at 50µm isotropic resolution (Figure 1). The olfactory bulb image acquired by the first echo is T_1 weighted and shows the Mn^{2+} enhanced layers of the bulb. The next successive echoes in the sequence are more T_2* weighted and highlights the presence of MPIOs. MPIOs could be overlaid on the T_1 weighted image to form a composite image. From this composite image, the MPIOs could be counted with layer specificity in the olfactory bulb. Olfactory bulbs from naïve and odor exposed animals were compared using flow cytometric analysis and MRI. The FACS analysis showed that there was no significant difference between naïve and odor enriched animals in the number of GAD67+, tyrosine hyrdoxylase+, and MPIO+ cells. Though the number of cells was similar, MRI showed that the distribution within the olfactory bulb differed as demonstrated in Figure 2. There was no significant difference in MPIOs counted in the glomerular (GL) or external plexiform layer (EPL) but there were twice as many MPIOs counted in the mitral cell layer (MI) of odor enriched animals compared to naïve animals.

Discussion

Manganese enhanced MRI can be utilized with MPIOs to identify NPCs in the olfactory bulb with layer specificity. Odor enriched animals had twice as many MPIOs compared to naïve animals in the mitral cell layer. In addition, a high concentration of MPIO+ cells was identified in the MI layer in the same region where the odor amyl acetate exposure was found to activate the glomerular layer [7]. These NPCs in the mitral cell layer may be necessary in olfaction for the purpose of modulating the sensory signal from the glomeruli and work is ongoing to identify the exact nature of these MPIO labeled cells. Combining functional MRI approaches with the cell labeling approaches demonstrated in this work should enable determining whether these new cells affect odor processing.

References

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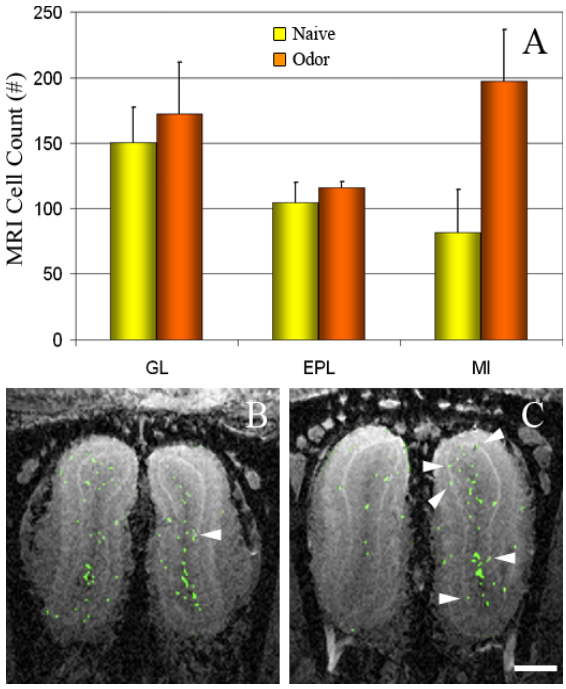

Figure 2: MRI measurement of new neurons in the olfactory bulb. Panel A shows the number of MPIOs counted in each layer of the olfactory bulb. Only the mitral cell layer shows a significant difference ($p = 0.01$). An olfactory bulb MRI from a B) naïve and C) odor exposed animal. Arrows indicate MPIOs in the mitral cell layer. Scale bar = 1 mm. ($n_{naive} = 3$, $n_{odor} = 5$)