In Vivo Labeling of Adult Neural Progenitors with Micron Sized Particles of Iron Oxide: Quantitation of Labeled Cell Type

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Introduction

Neurogenesis in the olfactory bulb occurs throughout adulthood in rodents[1]. Migrating neural progenitors originate at the ventral side of the lateral ventricle, known as the subventricular zone. The cells migrate through a well defined pathway called the rostral migratory stream (RMS) from the SVZ to the olfactory bulb. We are interested in monitoring the migration of these neural progenitors to map out the migration pathway throughout the rat forebrain. Previously we demonstrated that migrating precursors could be visualized with MR after in vivo labeling with micron sized iron oxide particles (MPIOs) that were injected into the lateral ventricle[2]. Here we extend these studies to quantify the cell types that get labeled and prove that the MRI contrast that develops along the RMS requires migrating precursors. High resolution, gradient-echo MRI was used to monitor the neural progenitors' migration to the olfactory bulb. Immunocytochemistry (ICC) and immunohistochemistry (IHC) were performed to quantitate the cell types that the MPIOs were labeling. Furthermore, treating animals with Ara-C in a way that is known to eliminate endogenous neural stem cells did not lead to MRI contrast along the RMS, conclusively demonstrating that MRI can detect migrating cells after in vivo labeling with MPIOs.

Materials and Methods

Neural progenitor cells were labeled in vivo by stereotactic injection of 50 μL of 1.63 μm MPIOs into the lateral ventricle of (AP: -2, ML: +2, from the bregma, and DV: -3 from the dura). The MPIOs were polystyrene coated iron oxide particles that contain Dragon Green, a FITC derivative (Bangs Laboratories, Fishers, IN, USA). Additionally, 10 rats were also continually infused with 4% cytosine β-D-arabinofuranoside (Ara-C), an anti-mitotic agent, for four weeks after particle injection using implanted osmotic pumps. Ara-c has been shown to eliminate neural stem cells following infusion[3], and thus served as a control for particle migration. 3-D Flash gradient echo images were acquired on anesthetized rats over a 4-week period (scanning every 2 weeks) with the following imaging parameters: TR: 8.0 ms, TE: 30 ms, 100 μm isotropic resolution (FOV of 2.56 cm^3). MRI imaging data was collected on an 11.7 T magnet with a volume transmit and a 2 cm receive coil. To determine what cell types particles were in, cells were recovered from injected rat brains and processed for ICC. Cells were isolated from rat brains by first decapitating anesthetized animals. The brain was then removed and minced into small pieces before being placed in a 20 U/mg papain solution for 1 hour at 37°C. The papain solution was triturated several times then placed on a Percoll gradient to remove debris. Cells isolated from the gradient were prepared for fluorescence activated cell sorting (FACS) analysis using ICC. Only live cells were analyzed with FACS. Magnetically activated cell sorting (MACS) was also used to separate cells containing particles, which were then fixed in 4% parafomaldehyde and stained for ICC using both surface and cytoskeletal epitopes. Antibodies used for ICC and IHC include anti-MAP2 (neurons), anti-GFAP (astrocytes), anti-O4 (oligodendricytes), anti-Iba 1 (microglia), and anti-vimentin (early precursor cell). Brains were also prepared for IHC. Rats were perfused and fixed with a 10% formalin buffered solution, and the brains were removed with the olfactory bulbs intact. Identical primary and secondary antibodies were used as for the ICC preparation.

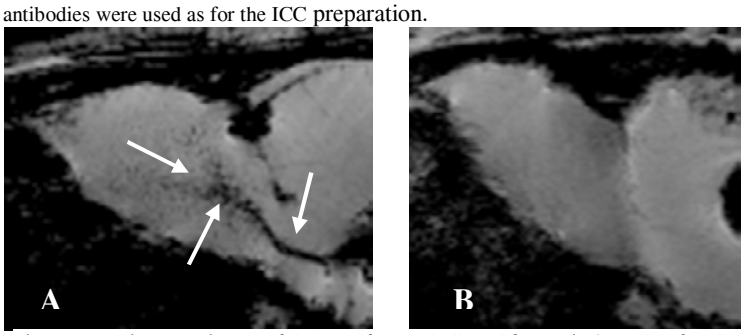

Figure 1: In vivo MRI image of the rat olfactory bulb and forebrain 2 weeks after A) MPIO injection and B) MPIO injection and Ara-C infusion into the lateral ventricle. Dark contrast from the MPIO is found both in the rostral migratory stream and olfactory bulb (arrows). Ara C eliminates this contrast due to killing of progenitors.

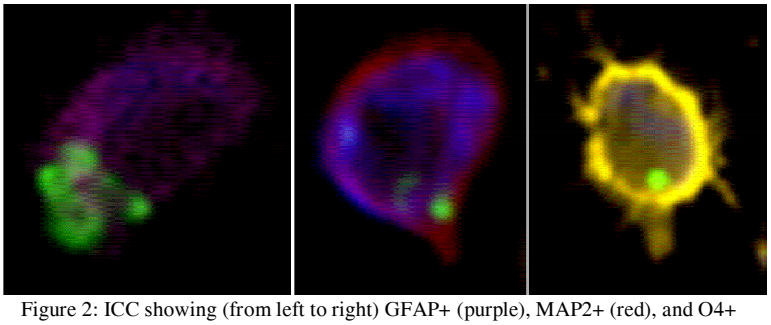

Figure 2: ICC showing (from left to right) GFAP+ (purple), MAP2+ (red), and O4+ (yellow) cells with MPIOs (green) and vimentin (blue) that were isolated using MACS

Results

Figure 1 shows in vivo MR images of the rat forebrain and olfactory bulb 2 weeks after A) injections with MPIO and B) injection of MPIO with the infusion of Ara-C. Migration of the MPIOs caused hypointense contrast to be observed in the RMS and olfactory bulb. This contrast was eliminated by infusing the anti-mitotic agent, Ara-C (n=7). Fluorescence microscopy of ICC recovered cells and IHC prepared brain sections showed that the MPIOs were contained in the three major cell types of the brain, astrocytes, oligodendricytes, and neurons. The particles were also found in microglia. Figure 2 shows fluorescent images of individual cells recovered from the rat brain using MACS. These cells contained MPIOs and were vimentin positive, indicating that they were neural progenitor cells. Of all the cells that contained MPIOs, 59% of GFAP+, 15% of MAP2+, and 37% of O4+ cells also co-stained with vimentin. Cells that were vimentin negative were also isolated. IHC confirmed the presence of vimentin positive cells in the RMS, including neurons, astrocytes, and oligodendricytes. MPIOs identified in the olfactory bulb were only found in cells that were positive for the neural marker, MAP2. This indicated that once the cells left the RMS and entered into the olfactory bulb, they differentiated into neurons. In the rat forebrain and olfactory bulb, 32% of neurons isolated, 41% of astrocytes isolated, and 23% of oligodendricytes isolated for FACS analysis contained MPIOs.

Discussion

MPIOs can be incorporated into migrating neural precursors by direct injection of free particles into the ventricle, and these cells can be observed using MRI. The migration can be inhibited using an anti-mitotic agent Ara-C which kills the neural progenitors[3]. This proves that particles can not move by themselves, and only migrate with neural precursors. Environmental stimuli may affect the cell distribution within the rodent forebrain and could potentially be visualized using MRI and compared with IHC and ICC.

References

1) Abrous, D.N., et al., *Physiological Reviews* 85(2), 523-569 (2005); 2) Shapiro, EM, et al, *NeuroImage* 32(3), 1150-7 (2006); 3) Doetsch, F., et al., *PNAS* 96(20), 11619-24 (1999).