

MRI of Neural Stem Cell Migration in the Adult Mouse Brain

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Introduction:

The use of MRI to observe endogenous neural progenitor cell migration in the mouse brain may greatly aid the study of neural stem cell (NSC) response to brain injury and disease. In the adult mouse, NSCs of the subventricular zone (SVZ) differentiate and migrate along the rostral migratory stream (RMS) to become interneurons of the olfactory bulb (OB). Endogenous NSCs of the SVZ have also been observed to proliferate, differentiate, and migrate towards areas of neurodegeneration and injury, including excitotoxic injury¹.

Iron-oxide particles, a favored agent for cellular MRI, have previously been used to label endogenous NSCs *in situ* in rats², and efforts have been made in mice³. An earlier study by Shapiro *et al.* required that large numbers of micron-sized particles of iron-oxide (MPIOs) be injected into the lateral ventricle (LV), resulting in a large hypointense region on MRI at the site of injection². Since NSC migration in response to injury may be restricted to relatively short distances, a large hypointense artifact at the injection site may obscure this localized migration. By injecting MPIOs into the SVZ directly, we have been able to reproduce the labeling of RMS neuronal progenitors in mice, and reduce the size of the hypointensity region by using a smaller volume of iron-oxide particles.

Methods:

6-8 week old female ICR mice were injected with 50 nL MPIOs (Bangs Laboratories) into the SVZ (stereotaxic coordinates +1.0 mm rostral to bregma, +1.0 mm lateral to bregma, -2.5 mm deep to dura), or with 2 μ L MPIOs (scaled roughly to the 50 μ L used previously in rats²) into the lateral ventricle (+0.2 mm rostral to bregma, +0.8 mm lateral to bregma, -2.5 mm deep to dura) using beveled glass needles and a microinjector. MPIOs have a 1.63 μ m mean diameter and fluoresce green, enabling immunofluorescent analysis. Mice were anesthetized with isoflurane and imaged *in vivo* using a 7T μ MRI (SMIS, Bruker). A T2*-weighted, 100 μ m, isotropic 3D gradient echo protocol was used (TE = 8 ms, TR = 50 ms, flip angle = 20°, 1 hour 50 minute scan time). Mice were scanned at 1.5-2, 3, 7, and 11 weeks post-injection.

Following imaging, mouse brains were perfused with paraformaldehyde and frozen for sectioning. Immunohistochemistry was performed using the following antibodies: goat anti-doublecortin (Santa Cruz) for immature, migrating neurons, and rabbit anti-Iba1 (Wako) for microglia.

Results:

After SVZ injection of MPIOs, we were able to observe the RMS, which appears hypointense on MRI (n = 15) (Figure 1A, yellow arrow; Figure 2). By 1.5-2 weeks post-injection, migration of NSCs was already robust, and cells could be seen dispersing throughout the ipsilateral OB (red arrow). The RMS was also seen on MRI after LV injection of MPIOs. (Figure 1B); larger volumes of MPIOs were needed for NSC labeling via LV injection, creating a larger hypointensity artifact around the injection site.

RMS labeling persisted at least 11 weeks after injection. Observation and registration of multiple brain images at early and later time points revealed that on average, hypointense particles are more widely distributed within the OB at later time points. In no animals was labeling seen on the contralateral side (data not shown).

Immunohistochemistry revealed that fluorescent MPIOs were co-localized in doublecortin-positive cells along the RMS and OB, indicating that MPIOs were within migrating neuroblasts. Immunohistochemistry was also performed to confirm that MPIOs were directly injected into the SVZ. Iba1 staining found no MPIOs in microglia (data not shown).

Conclusion and Discussion:

Injection of MPIOs directly into the SVZ resulted in labeled NSCs that were observed to migrate along the RMS to the OB. Direct injection of MPIOs into the SVZ allowed us to use a smaller volume of iron-oxide particles, resulting in a smaller hypointensity region on MRI. This method will allow us to label and track NSCs in many brain injury and disease studies.

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References:

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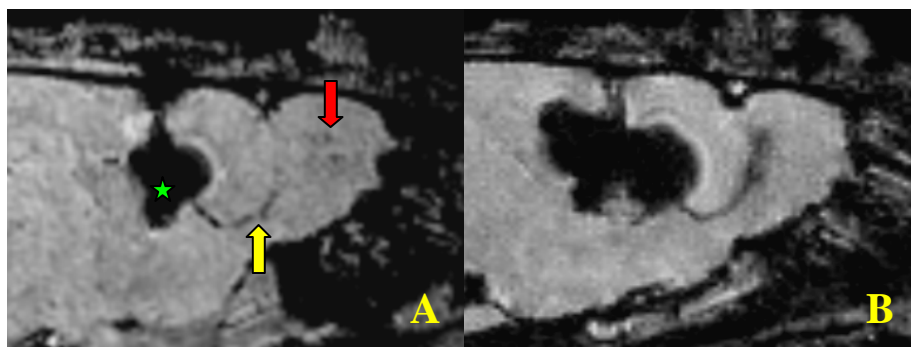


Figure 1. Migration of MPIOs along the RMS (yellow arrow) into the OB (red arrow). 2 weeks post-injection. Green star corresponds to injection site. (A: SVZ injection. B: LV injection).



Figure 2. Migration of MPIOs on a curvilinear plane along the RMS, showing the path of the migrating particles.