MRI of Neural Progenitor Migration in the Developing Mouse Brain

A. E. Deans[1], Y. Z. Wadghiri[1], D. H. Turnbull[1]
[1]New York University School of Medicine, New York, New York, United States

Introduction. Iron oxide contrast agents have been established in recent years as cellular contrast agents in the mouse adult [1-4] and embryo [5]. However, this approach has not been reported in studies of mouse neurodevelopment. Micron-sized particles of iron oxide (MPIO) are efficiently taken up by stem cells in culture [6] as well as *in situ* by neural progenitors in the adult subventricular zone [7]. In this study, we have used MPIO to label forebrain interneuron progenitors in the mid-gestation medial (MGE) and lateral (LGE) ganglionic eminences. It has previously been reported that MGE and LGE progenitors migrate tangentially to the forebrain cortex and to the striatum, respectively [8,9] during mid- to late-gestation. Our goal was to determine the feasibility of investigating these neural cell migrations using MRI.

Methods. For *in situ* progenitor cell labeling, green fluorescent MPIO (0.96μm diameter, Bangs Laboratories) were injected into the MGE or LGE of 13.5 day (E13.5) embryos (Fig.1a) through a 50μm diameter beveled glass needle using high frequency ultrasound biomicroscopy (UBM) for localization (Fig.1b). Embryos were harvested for *ex vivo* MRI immediately after injection (E13.5) or five days later (E18.5). At E13.5, embryos were fixed for 1 hour in 4% PFA, and at E18.5, embryos were cardio-perfused with cold 4% PFA and post-fixed for 1 hour. Embryo heads were mounted in a syringe phantom surrounded by Fomblin perfluoropolyether (Ausimont). T2*-weighted 7T-MRI demonstrated the location of particles within the embryonic brain: 3D gradient echo, TR/TE= 50ms/ 15ms, matrix=512^3, field-of-view=$(25.6\text{mm})^3$, flip angle=18°. Following imaging, E18.5 embryo heads were sectioned for histology. The locations of particles on overlayed fluorescence micrographs from 3 sequential 20μm thick sections were correlated with regions of signal loss from the matched 50μm MRI slice. Immunohistochemistry was done to identify the cell types which had been labeled with particles, using antibodies to Tuj-1 and PDGF-Rα to identify neurons and oligodendrocytes, respectively.

Results. T2*-weighted imaging demonstrated good localization of particles to the MGE (Fig.1c) or LGE (not shown) immediately after injection (E13.5 MRI). MRI at E18.5 showed particles dispersed throughout the striatum and cortex following MGE injection (Fig.1d) and restricted to the striatum following LGE injection (Fig.1e). Correlation between histology and MRI showed good general agreement between regions of signal loss (Fig.2a) on MRI and fluorescence microscopy (Fig.2b). On histologic analysis, cells labeled with particles following either MGE or LGE injection are Tuj-1 positive (Fig.2c) and PDGF-Rα negative (Fig.2d), indicating that labeled cells are neurons and not oligodendrocytes.

Conclusions. Progenitor cell migration from the MGE and LGE, following *in situ* cell labeling with MPIO at E13.5, was consistent with previous neurodevelopment studies [7,8]. *Ex vivo* MRI and histology of the embryos at E18.5 showed that some migrating MGE and LGE interneurons were labeled *in situ* with MPIO and detected on MRI. These results provide motivation for future *in vivo* MRI studies of neural cell migration in the developing mouse embryo.

References.
[1] Bulte, J. W., et al. (1999). Proc Natl Acad Sci **96(26)**: 15256-61.
[2] Bulte, J. W., et al. (2001). Nat Biotechnol **19(12)**: 1141-7.
[3] Hoehn, M., et al. (2002). Proc Natl Acad Sci **99(25)**: 16267-72.
[4] Bulte, J. W., et al. (2003). Magn Reson Med **50(1)**: 201-5.
[5] Shapiro, E. M.,et al. (2004). Proc Natl Acad Sci **101(30)**: 10901-6.
[6] Hinds, K. A., et al. (2003). Blood **102(3)**: 867-72.
[7] Shapiro, E.M, et al. (2004). Proc ISMRM: 166.
[8] Anderson, S. A. et al. (2001). Development **128(3)**: 353-63.
[9] Wichterle, H., et al. (2001). Development **128(19)**: 3759-71.

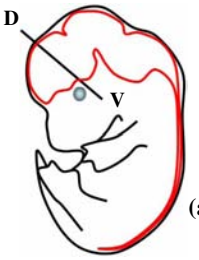

Fig.1. *In situ* labeling of interneuron progenitors. (a) Schematic of an E13.5 embryo (D=dorsal, V=ventral) with MGE and LGE as shown. (b) UBM image of localized injection into the MGE. (c)T2*-MRI at E13.5 showing injection site (*). MRI of fixed E18.5 embryos five days post-injection of MPIO into the MGE (d) or LGE (e). Widely dispersed migrating cells were observed following MGE injection (▲), but not following LGE injection.

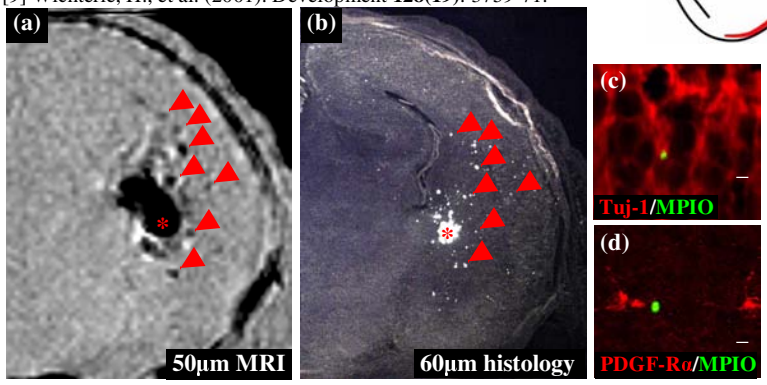

Fig.2. Correlation of MRI with histology. (a) 50μm isotropic MRI and (b) overlayed fluorescence micrographs of 3 x 20μm histologic sections from the matched sections of an MGE injected embryo (*: injection site; ▲: MPIO). Immunohistochemistry shows MPIO (green) within neurons (Tuj-1= red) (c), but not within oligodendrocytes (PDGF-Rα= red) (d) (bar=10μm).