# In vivo imaging of endogenous neural stem cells labelled with a ferumoxide-polycation complex using a low field system

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**Introduction.** A population of adult neural stem cell (NSC) resides in the subventricular zone (SVZ) of the lateral ventricles. NSC generate neuroblasts which migrate along the rostral migratory stream (RMS) toward the olfactory bulb (OB), where they differentiate into neurons (Doetsch et al 1999). Ferumoxide-based MRI contrast agents have been used to track the migration of exogenously-labelled NSC (Hoehn et al, 2002), and the protamine sulphate-ferumoxide complex (FePro), has been used to enhance ferumoxide labelling in vitro (Arbab 2005). To date, there are limited cell tracing methods that allow for longitudinal tracing of endogenous neural stem cells using MRI, and these depend on high-field imaging systems and large volume injections of contrast agent into the SVZ, which produces large susceptibility effects around the injection site (Shapiro, 2006). In this study, two MRI contrast agents, Endorem and FePro, were used to label the endogenous population of NSC and neuroblasts in situ in the SVZ by microinjection into the lateral ventricles, and cell labelling and MRI contrast were assessed up to 28 days post-labelling using a low-field system (2.35T). Cell migration was observed in the RMS *in vivo* and *ex vivo*, and histology confirmed labelling of migrating neuroblasts.

**Methods.** Animals were anaesthetised and placed on a stereotactic frame. Animals were injected with 2µl FePro or endorem into the left lateral ventricle. Animals were imaged at 7 days or 28 days then transcardially perfused fixed with 4% paraformaldehyde for ex vivo imaging and histology. Animals were scanned on a 2.35T system, with 2D coronal spin echo and 3D gradient echo sequences. In some animals, ex vivo 3D gradient echo images were acquired. Histological sections were stained with Prussian blue for iron detection and with Hematoxylin/Eosin, or for immunohistochemistry with doublecortin, nestin, and GFAP.

**Results.** Following Endorem injection, Endorem diffused outside of the lateral ventricle in all animals (n=3). Hypointensity in both ventricles was observed, as well as in the septum at one week post-injection (Fig). Prussian blue histology showed iron in the ventricular walls, the corpus callosum and the septal nuclei, as well as in the cortex along the injection tract (Fig). FePro injection produced hypointensity which was restricted to the left lateral ventricle in MRI images in all animals (n=12) (Fig), and was not present in the septum or right lateral ventricle. At four weeks post-injection, hypointensity in the RMS was observed *in vivo* and *ex vivo* but not in the OB (Fig). FePro-labelled cells were detected in the SVZ, RMS and OB granule cell layer with Prussian blue stain (Fig), demonstrating that FePro labelled cells are also present in areas where FePro is not detectable on MRI images. No iron was observed in the septum or outside of the RMS. Stereological quantification of cell labelling at four weeks post-injection determined 200 labelled cells per animal in the RMS (n=6). Doublecortin immunohistochemistry co-labelled with Prussian blue staining, which indicated that neuroblasts, which are derived from the SVZ, had incorporated the FePro label (Fig).

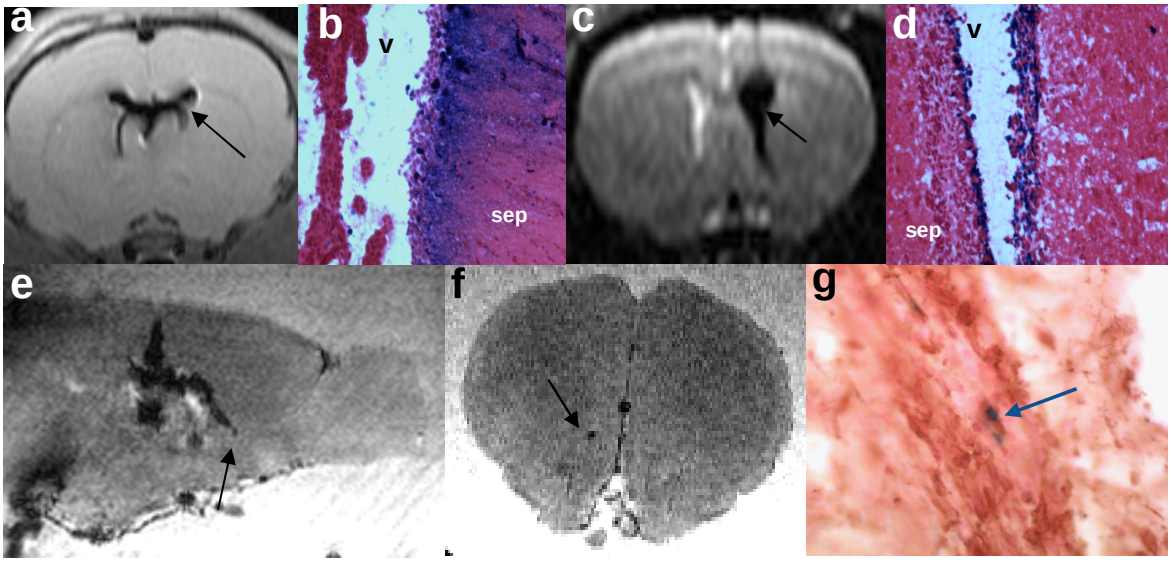


**Figure 1.** A, Endorem in the septum and contralateral ventricle at 7 days post-injection. Arrow, injection site. B, Prussian blue stain for iron demonstrates Endorem in the septum on histology. sep, septum; v, ventricle. C, FePro is restricted to the injected ventricle at 7 days post-injection (arrow). D, Prussian blue stain for iron demonstrates FePro is restricted to the SVZ. E, F, Sagittal (E) and coronal (F) ex-vivo images shows FePro in the left RMS (arrow). G, Doublecortin immunostaining and Prussian blue show FePro-labelled neuroblast in the RMS (arrow).

**Conclusion.** These results demonstrated that the distribution of Endorem and FePro MRI contrast agents are different when injected into the lateral ventricle; FePro is restricted to the ventricles and Endorem diffuses into the septum. The FePro contrast agent labelled cells in the SVZ, of which a percentage generated neuroblasts that migrated through the RMS to the olfactory bulb, and this migration could be imaged *in vivo* and *ex vivo*. We conclude that FePro is an appropriate MRI contrast agent for in situ longitudinal cell tracking studies. This study demonstrates that small volumes of ferumoxide can efficiently label cells in the SVZ, and migrating cells can be detected away from the injection site on low field strength systems. *In vitro* cell labelling with FePro requires less contrast agent for equivalent cell iron loading compared to Endorem (Arbab et al, 2005; and unpublished data). Previous studies labelling endogenous neural stem cell with iron oxide contrast agents have used high-field systems and large volumes of contrast agent in the SVZ to achieve cell detection, with significant susceptibility effects around the injection site (Shapiro et al, 2006). While cell detection outside of the SVZ is less compared to previous studies, susceptibility at the injection site is also reduced. This is important for cell tracking in models of disease, where neuroblast migration distances are shorter than the long-distance migration to the OB. This cell tracking method will be used to assess the migration of endogenous NSC toward lesion sites following cerebral ischaemia.

**References**

Doetsch F, et al. Cell. 1999 Jun 11;97(6):703-16.
Hoehn M, et al. Proc Natl Acad Sci U S A. 2002 Dec 10;99(25):16267-72.
Arbab AS, et al. NMR Biomed. 2005 Dec;18(8):553-9.
Shapiro EM, et al. Neuroimage. 2006 Sep;32(3):1150-7.