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Magnetic resonance imaging of the migration of neuronal precursors generated in the adult rodent brain

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Neural progenitor cells (NPCs) reside within the subventricular zone (SVZ) in rodents. These NPCs give rise to neural precursors in adults that migrate to the olfactory bulb (OB) along a well-defined pathway, the rostral migratory stream (RMS). Here we demonstrate that these NPCs can be labeled, in vivo, in adult rats with fluorescent, micron-sized iron oxide particles (MPIOs), and that magnetic resonance imaging (MRI) can detect migrating neural precursors carrying MPIOs along the RMS to the OB. Immunohistochemistry and electron microscopy indicated that particles were inside GFAP$^+$ neural progenitor cells in the SVZ, migrating PSA-NCAM$^+$ and Doublecortin$^+$ neural precursors within the RMS and OB, and Neu-N$^+$ mature neurons in the OB. This work demonstrates that in vivo cell labeling of progenitor cells for MRI is possible and enables the serial, non-invasive visualization of endogenous progenitor/precursor cell migration.

Keywords: MRI; Iron oxide; Stem cells; Neurons; Contrast agents

Introduction

The subventricular zone (SVZ) is the largest germinal layer in the adult rodent brain. It is localized next to the lateral wall of the lateral ventricle. Primary progenitors in this region correspond to type B cells, which have properties of astrocytes (Garcia-Verdugo et al., 1998; Doetsch et al., 1999). These cells divide to generate transit amplifying type C cells, which generate new neurons that migrate to the olfactory bulbs (OBs) (Garcia-Verdugo et al., 1998; Doetsch et al., 1999) along a well-defined pathway, the rostral migratory stream (RMS) (Doetsch and Alvarez-Buylla, 1996). Once in the OB, these new neurons differentiate into granule cell neurons and periglomerular neurons (Lois and Alvarez-Buylla, 1993). In mice, many new neurons reach the OB by two days and a majority arrive in the OB by day 6 (Lois and Alvarez-Buylla, 1994). Experimentally induced traumatic brain injury (Salman et al., 2004) and stroke (Zhang et al., 2004) induce new SVZ progenitor cell proliferation and migration, suggesting that these cells may also have a function in repair mechanisms. Additionally, analogous neural progenitor cells have recently been discovered in non-human primates (Kornack and Rakic, 2001; Pencea et al., 2001) and in humans (Sanai et al., 2004).

To date, fluorescence imaging and electron microscopy (EM) have been principally used to identify migrating cells derived from cells in the SVZ. Due to the number of antibodies specific to these cells, immunohistochemistry has been used to detect groups of cells in well-defined areas. Bromodeoxyuridine has also been used to specifically label these cells because they divide in the SVZ before migrating. Additionally, transgenic cells expressing green fluorescent protein have been successfully used to study cell migration (Suzuki and Goldman, 2003). However, none of these techniques enable migration throughout the brain within living animals to be studied.

Magnetic resonance imaging (MRI)-based neural cell tracking in intact animals was first described in 1990 (Ghosh et al., 1990) and has been further developed in a number of laboratories (Tang et al., 2003; Hoehn et al., 2002; Bulte et al., 2001; Franklin et al., 1999; Yeh et al., 1995; Hawrylak et al., 1993; see Bulte and Kraitchman, 2004, for a recent review of this field). In nearly every demonstration of cell tracking by MRI, cells were labeled with an MRI contrast agent in culture,

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injected into the animal, and imaged either in vivo or in vitro. Only blood borne cells such as macrophages have been consistently labeled with MRI contrast agent in vivo (Zhang et al., 2000). The MRI contrast agent most often used is a nanometer-sized dextran-coated iron oxide nanoparticles (commonly referred to as USPIOs or MIONs, 30–200 nm diameter). An advantage of iron oxide particles as an MRI contrast agent is that iron oxide disturbs the otherwise homogeneous magnetic field used for MRI, and as such cells harboring iron oxide generate dark spots in MRI (Lauterbur et al., 1986). The large quantity of iron oxide that can be loaded into cells, primarily by endocytosis, enables sensitive detection of cells. A major advantage of using MRI to study cell migration is that the migration can be directly mapped onto the anatomy available from MRI, as well as functional activity available from fMRI or neuronal tract tracing (Pautler et al., 1998).

Recently, it has been demonstrated that micron-sized iron oxide particles (MPIOs) have some advantages for cell labeling studies by MRI (Shapiro et al., 2005). Very stable particles are readily available, which are impregnated with various fluorescent dyes. Most cells studied to date readily endocytose MPIOs. Each particle contains 1–10 pg of iron, enabling very high levels of iron loading. Indeed, even single MPIO particles have been detected in single cells in culture (Shapiro et al., 2005) and in fixed mouse embryos (Shapiro et al., 2004). Furthermore, single cells labeled with MPIOs have been detected, in vivo, by MRI. This was accomplished in mouse livers with transplanted hepatocytes (Shapiro et al., 2006) and in rat hearts with individual macrophages (Wu et al., 2005). Thus, MPIOs open the possibility of sensitive labeling and detection of cells for MRI.

In principle, as few as a single MPIO is needed to be taken up by cells to be labeled for MRI. Therefore, it should be possible to directly label cells in vivo. The goal of the present work was to label neural progenitor cells in the SVZ, in vivo, by direct injection of MRI contrast agent into the lateral ventricles, near the SVZ. Due to the small size of the SVZ in adult rats and subsequent difficulty in consistently delivering injections there, the strategy was to target the neural progenitors via the ventricle. As only single MPIOs are required for detection of cells (Shapiro et al., 2004), inefficient labeling could be tolerated. Following endocytosis of the particles from the ventricle, the particles were incorporated into daughter neural precursors through cell division. When the neural precursors migrated along the RMS, the presence of one or more MPIOs generated dark spots in the MRI revealing the locations of the migratory cells. Immunohistochemistry and Prussian blue iron staining confirmed the presence of particles in SVZ cells close to the lateral ventricle and along the RMS, and in mature neurons in the granule cell and periglomerular layers. EM confirmed the intracellular location of the particles in granule cell neurons in the OB.

Materials and methods

Animal injections

Twenty, 6-week-old Sprague–Dawley rats (Harlan, Indianapolis, Indiana) were stereotactically injected with 5–50 μl (1.5×10^7 to 1.5×10^8 particles) of 1.63 μm diameter MPIOs (encapsulated, fluorescent, magnetic beads, Bangs Laboratories, Fishers, IN) into the anterior right lateral ventricle. The coordinates chosen were 2 mm caudal from bregma, right 2 mm, down 3 mm, and were based on prior mapping of rat cerebral anatomy in similar sized rats. The MPIOs were styrene/divinylbenzene-coated iron oxide microparticles with a green fluorescent dye (480 excitation, 520 emission, standard green fluorescent protein filters) impregnated into the shell. The MPIOs were COOH−functionalized and contained 45% w/w iron oxide. In some cases, 400 μl MPIOs was soaked overnight with 100 μg EGF (Sigma) prior to injection. As the RMS exists proximal to a white matter band, control ($n = 3$) injections were also performed by injecting 50 μl (1.5×10^9 particles) of MPIOs into the corpus callosum. This served to test for background migration along white matter tracks. Six animals were also injected with 50 μl (1.5×10^9 particles) of MPIOs into the cortex near the SVZ, serving as a control for gray matter migration. All experiments were carried out in compliance with guidelines set by the National Institute of Neurological Disorders and Stroke Animal Care and Use Committee.

MRI

Immediately following injection, animals underwent a baseline MRI investigation. MRI was performed either on an 11.7-T or a 7.0-T system (Bruker Biospin, Billerica, MA, Paravision software 3.0.1). Separate transmit-only volume (35 mm birdcage coil) and receive-only surface coils (30 mm diameter) were used. Under 3% isoflurane anesthesia in 90% oxygen/10% medical air, animals were placed in a custom-built MR compatible stereotactic head frame. The high oxygen concentration lowers the amount of deoxygenated blood, lessening the appearance of dark veins in the MR images (Dunn et al., 2002). The surface coil was placed directly on the head of animals. Animals were orally intubated and mechanically ventilated at 65 breaths/min and end tidal CO_2 and respiratory patterns were monitored. Body temperature was maintained at 37° C by use of a circulating water bath and rectal temperature feedback. 3D gradient echo MRI was performed using the following image parameters: FOV 2.56 cm^3, matrix 256^3 (100 μm isotropic voxel size), TR = 70 or 30 ms (75 or 32 min acquisition), TE = 8 ms, 12.5 kHz acquisition BW. Following MRI, animals were revived and returned to the animal facility. Subsequent MRI was performed either weekly for 4 weeks ($n = 7$) or once at 5 weeks post-injection ($n = 13$).

Following the last MRI session, animals were transcardially perfused with 0.9% saline, followed by 10% formalin containing 1 mM Gd-DTPA. The Gd-DTPA reduces the T_1 of the sample to allow rapid imaging. Brains, with intact olfactory bulbs were removed and post-fixed overnight in the same Gd-DTPA containing fixative. Brains were then placed in saline and imaged in vitro in a 35-mm birdcage coil with the following image parameters: gradient echo MRI, FOV $2.56 \times 1.28 \times 1.28$ cm^3, matrix $512 \times 256 \times 256$ (50 μm isotropic voxel size), TR = 70 ms, TE = 8 ms, 12.5 kHz acquisition BW, 16 averages.

Histology

For immunohistochemistry, brains were processed for frozen sections. Frozen sections preserve the fluorescence on the