Serial monitoring and quantification of endogenous neuroblast migration rates by cellular MRI

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INTRODUCTION: *In vivo* labeling of endogenous neural progenitor cells in adult rat brains is an established method for tracking native cell migration vivo (1-2). Briefly, micron sized iron oxide particles (MPIOs) are injected into the lateral ventricle proximal to the neural stem cell niche in the brain. MPIOs are endocytosed and incorporated into the neural progenitor cell population, making them visible by susceptibility weighted gradient echo MRI. Here we extend this new method to quantifying cell migration rate and numbers over time. Previous to this study, the only other method for acquiring this information was to sacrifice animals and perform immunohistochemistry on pooled samples. Using MRI to acquire this information allows one to reconstruct migration trajectories, perform serial investigations on the same animal and provides a means for translating this or similar experiments to primates.

Initially, we optimized *in vivo* cell labeling methodologies as high susceptibility effects from the MPIOs generate substantial signal loss, masking early migratory events, particularly around the injection site. Using optimized labeling conditions, we next conducted a longitudinal study over two weeks to quantify the migration of labeled progenitor cells towards the olfactory bulb (OB). To quantify cell migration from MR images we employed Bioimage Suite software program (3). This program facilitates determination of dark signal voids by simultaneously projecting three orthogonal imaging planes, highlighting determined signal voids in all planes. These MRI results are in accordance with those from purely immunohistochemical studies and represent the first use of MRI to temporally quantify endogenous neural progenitor cell migration to the OB.

MATERIALS AND METHODS:

In vivo labeling: To determine the optimal dose of 1.63 µm MPIOs to enable monitoring of early neuroblast migration along the rostral migratory stream (RMS) and OB, various quantities of green fluorescent MPIOs at $3*10^9$ MPIOs/ml (5, 10, 20 or 50 µl total, n=6 for each group) were stereotactically injected into the anterior ventricle proximal to the subventricular zone (SVZ) in adult (6 wk old, 150 g) Sprague-Dawley rats. 3D gradient echo images (TE = 10 ms, TR = 30 ms, 100 μm^3 resolution) were acquired at 11.7T at 1, 3, 8 and 14 days post injection.

Immunohistochemistry: Rat brain sections (16 µm) were stained for doublecortin, a marker for migrating progenitor cells; IBA1, a marker for microglia; GFAP, a marker for ventricular astrocyte-like stem cells and NeuN, marker for mature neurons.

Data analysis: 3D data sets from the 20 µl injected animals only were imported into BioImage Suite. First a rigid linear registration was applied to all brains according to a predetermined template. Neuronal precursor cell migration from the SVZ, along the RMS to the OB was serially quantified by detection of dark signal voids in the OB. Using phantom samples we previously demonstrated that single cells labeled with 1.63 µm MPIOs produce a 30% drop in signal intensity in a gradient echo image (4). Thus, we have defined pixels containing labeled cells by applying a threshold of > 30% decrease in signal intensity relative to the background signal. The occupancy of labeled precursor cells in the OB (in mm^3 volume), was normalized to each OB and presented as volume percentage as a function of time following MPIO injection.

RESULTS: Dose dependent studies revealed an optimal labeling dose of 20 µl ($6*10^7$ MPIOs total). This was determined by taking into account the balance between visualization of early migratory events versus large susceptibility artifacts which obscure visualization of the early portion of the RMS caused by non-specific labeling of cells at the ventricle. Migration of MPIO labeled neural precursor cells was detected as early as 1 day post-injection of MPIOs into the ventricles, and at 2-3 days signal voids were detected in the OB (Figure 1). Accumulation of labeled cells in the OB not only increased during the first 8 days after injection, but also spread to the outer edges of the OB (Figure 2A). Immunohistochemical studies confirmed the presence of MPIOs within doublecortin positive neural progenitor cells in the OB. We quantified this accumulation over a period of 14 days following injection (Figure 2B). By 3 days following injection, we calculated 0.26 % of the volume of the OB contained labeled cells. By 8 days, this volume nearly doubled to 0.49%. At 14 days post injection, no difference was measured in the occupancy in the OB.

DISCUSSION: New paradigms in cellular therapies aim to employ reserves of endogenous cell populations. While numerous MRI methods exist for tracking transplanted cells, there is a similar need for methods which can not only monitor but quantify native cell migration. In this study we have tuned the *in vivo* cellular labeling protocol to enable visualization of early migration of cells into the OB. This facilitated quantification of this phenomenon. During the first week following labeling we measured a linear accumulation of newly arriving cells in the OB. Initially, at day 1, cells are confined to the RMS and are not found in the OB. By day 3, cells are observed entering the central portion of the OB; the granule cell layer, where most cells are destined to reside. At day 8, many cells can be detected not only in the central portion of the OB, but also in the outer edges, the periglomerular layer, where some cells can migrate to (1,5). MRI detection of migration plateaus from week 1 to 2, indicating that the MPIO injection acts like a bolus, not a sustained slow release label. This migration rate matches nearly identically to classical neurogenesis studies employing immunohistochemical means (5).

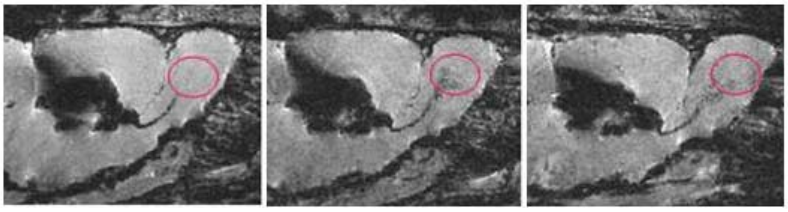

Figure 1: Early detection of endogenous neuronal progenitor cell migration. 3D GRE MR images acquired at 1 (left), 3.5 (middle) and 8 days (right) post injection.

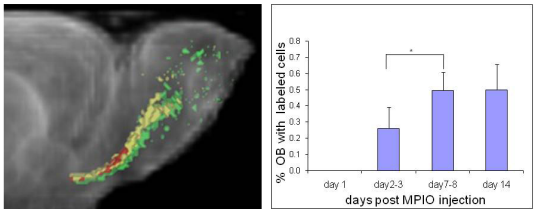

Figure 2: Quantification of MPIO labeled neuronal precursor cells in the OB. A) Overlay of serial progression of cell migration into the OB - red, 1 day; yellow, 3 days and green 8 days after injection of MPIOs. B) Quantification of volume increase of labeled precursor cell population in the olfactory. p value < 0.05.

References: 1. Shapiro, EM, *Neuroimage* (32) 2006, 2. Sumner, JP, et al, *J Biomed Optics* (12) 2007, 3. Papademetris, X, et al, http://www.bioimagesuite.org. 4. Shapiro, EM,*MRM* (53) 2005, 5. Luskin, MB, *Neuron* (11) 1993.