IN VIVO QUANTIFICATION OF PARTICLE BASED AND GENE BASED MRI REPORTERS IN THE RODENT BRAIN

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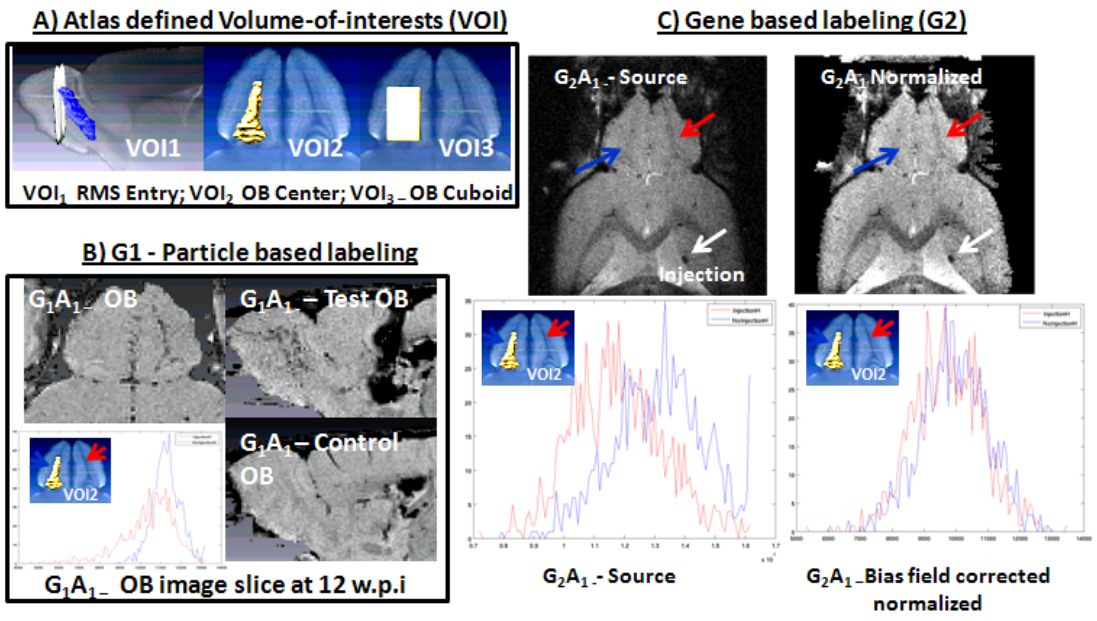
INTRODUCTION: MRI reporters for in vivo labelling and visualization of endogenous progenitor stem cells (eNPCs) in the adult rodent brain, such as iron oxide particles or reporter genes (e.g. ferritin), result in hypo-intense contrast in high resolution 3D T_2*-weighted MR images that can be quantified using suitable image processing tools. We have previously demonstrated that the assessment of lentiviral (LV) and adeno-associated viral (AAV) vector induced expression of the ferritin reporter gene with MRI is confounded by unspecific background contrast at the site-of-injection of the vector in the subventricular zone (SVZ) and around the injection tract (1). However, as the labeled eNPCs migrate away from the SVZ towards the olfactory bulb (OB), LV or AAV mediated MR reporter gene labeling of stem cells still holds potential for neural progenitor cell tracking with MRI. In (2), we quantitatively compared the use of ferritin reporter gene based and micron-sized iron oxide particle (MPIO) based labeling for visualization of the migrated cells in OB using high resolution *ex vivo* MRI. Here, we demonstrate the potential of this methodology for regional quantification of MRI reporter contrast from *in vivo* MRI, which could enable longitudinal analysis of regional particle based and reporter gene based MRI contrast changes over time in the same animal.

METHODS: 8 C57BL/6 mice (8-12 weeks old) were used in this study. A first group (G1, n=4) were injected in the right lateral ventricle with 2 µl MPIOs-PLL ($9.1 \cdot x\ 10^5$ particles, 0.67 mg Fe/ml), while a second group (G2, n=4) were injected in the right SVZ with LV encoded MRI reporter (LV-ferritin: LV-FerrH-IRES-FerrL). G1 was imaged with T_2*-w 3D-MGE MRI (78 µm isotropic resolution, 196x256x128) at 4, 6 and 12 weeks post-injection (w.p.i) and G2 with T_2*-w 3D MRI (80µm, 128^3 isotropic) at 3, 5, 10 and 30 w.p.i. on a 9.4T small animal scanner (Bruker Biospin, Ettlingen, Germany). Each of the 3D MR images (G1: 4x3=12 images; G2: 4x4=16 images) were corrected for RF intensity inhomogeneity and spatially normalized (affine) to an MR anatomical atlas template in Paxinos space using our image analysis pipeline (1,2). Three volumes of interest (VOI) were defined in the atlas template within the right OB (i.e. ipsilateral to the injection), namely the region where the rostral migratory stream (RMS) enters the center of the OB (VOI 1), the center of the OB (VOI 2), and a cuboid fully contained in OB (VOI 3), see Fig. A. Identical VOIs in the left OB (i.e. contralateral to the injection) were obtained by mirroring the original VOIs around the mid-sagittal plane of the atlas template. Corresponding right and left VOIs were subsequently identified in each of the study images by spatial transformation of the atlas VOIs. Inter-scan intensity variation (due to scanner drift, receiver gain etc.) was eliminated by normalizing the intensity values such that the mean and standard deviation within the left (i.e. control) VOI2 was identical for all images within the same group (G1, G2). The intensity values of the bias field corrected and normalized 3D T_2*-w images were compared within corresponding right (i.e. test) and left (i.e. control) VOIs 1, 2 and 3. Contrast differences between these VOIs were assessed group-wise by a paired t-test on the mean intensity of each VOI ($p<0.05$, n=12 for G1, n=16 for G2). In addition, contrast differences between these VOIs were assessed in individual animals for the last time point image (G1: 12 w.p.i.; G2: 30 w.p.i.) using an unpaired t-test on the intensity values of corresponding VOIs.

RESULTS: Fig. B illustrates the MPIO induced hypo-intense contrast in the right (test) OB of a representative case from G1, versus normal contrast in the left (control) OB, which is also apparent in the histogram (VOI3, right: red curve; left: blue curve). Fig. C shows an image slice and corresponding histograms (VOI3) for a representative case from G2, before (source) and after (normalized) bias field correction and intensity normalization. In comparison to MPIO, LV-ferritin labeling is more specific but the induced contrast is rather weak and also confounded by RF inhomogeneity and thus more subtle to detect. Group-wise comparison of the mean intensity showed a significant hypo-intense contrast difference of test versus control OB in both groups and for all 3 VOIs ($p<0.05$). For G1, all animals had significantly reduced MRI intensity in the test OB at the last time point ($p<<1e-6$). For G2, despite the fact that contrast differences could not be readily detected by visual inspection of the images, a significant difference ($p<0.01$) was found for all but one animal ($p>0.5$). For this particular case, hypo-intense contrast at the SVZ injection site was markedly absent compared to the other animals in G2, likely indicating an anomaly with the vector injection itself.



CONCLUSIONS: Our image analysis pipeline facilitates accurate and objective regional quantification of reporter induced hypo-intense MRI contrast in rodent brain by eliminating confounding effects of RF inhomogeneity, inter-scan variability and VOI selection. We have demonstrated its potential for assessment of both gene based and iron oxide particle based MRI reporter contrast from *in vivo* acquired MRI images. This may enable quantification of temporal changes in MRI contrast in longitudinal imaging studies, for instance to assess labeled cell migration over time.

REFERENCES

(1) G. Vande Velde in *ISMRM - ESMRMB Joint Annual Meeting, Stockholm* (2010)
(2) J.R. Rangarajan, et. al. Bi-annual meeting *Contrast agents and multi-modal molecular imaging, Mons* (2010)