CONTRAST QUANTIFICATION OF MPIO LABELED CELL MIGRATION IN THE OB OF MICE AS A READ OUT FOR THE EFFICIENCY OF IN SITU LABELING STRATEGIES

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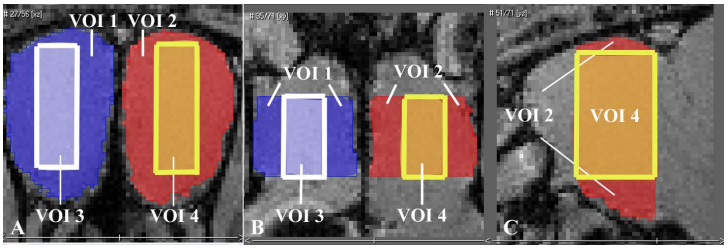
Introduction Visualization of endogenous neuronal progenitor cells (eNPC) migration with MRI has recently been described in adult rats[1] and efforts have been made in mice[2, 3]. We attempted to develop a method to measure contrast accumulation in the olfactory bulb (OB) as a tool to validate different *in situ* labeling strategies with MPIOs. The method is based on threshold segmentation of hypointense voxels, originating from MPIO-labeled eNPC. For contrast quantification, volumes of interest (VOIs; Figure 1: (A) coronal, (B) horizontal and (C) sagittal view) were manually outlined. Two rectangular VOIs comprising the core of the OB ipsilateral (VOI_3, light blue) and contralateral (VOI_4, orange) represent the regions where migrating eNPC enter the OBs. As general debiasing of the MRI did not work properly, two additional VOIs comprising the outer layers of the OB (ipsilateral: VOI_1, dark blue; contralateral: VOI_2, red) were added to correct for signal bias between the two OBs.

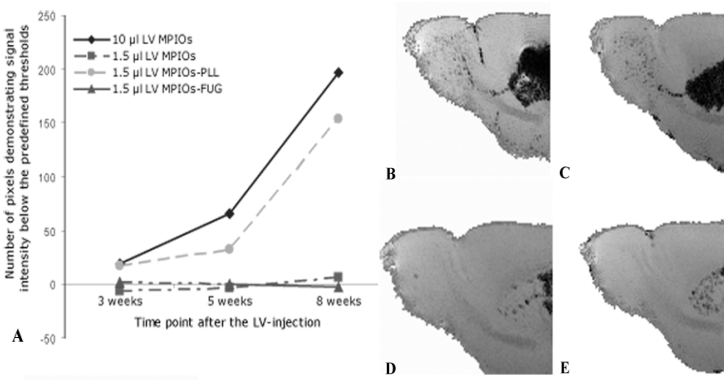
Figure 1



Methods Sixteen adult male mice (C57BL/6J) were stereotactically injected with 1.63 micron MPIOs (with green-fluorescent shell, iron oxide core; Bangs Laboratories, Inc) in the left lateral ventricle (LV). Four different injection strategies (n = 4 for all) were applied: 1) 10 µl MPIOs (3.00mg Fe/ml); 2) 1.5 µl MPIOs (0.67 mg Fe/ml); 3) 1.5 µl MPIOs (0.67 mg Fe/ml) combined with poly-L-Lysine (PLL) and 4) 1.5 µl MPIOs (0.67 mg Fe/ml) combined with Fugene-6 (FUG). At 3, 5 and 8 weeks post injection, one mouse of each group was perfused and decapitated for high-resolution (66 µm isotropic) *ex vivo* 3D-GE MRI (7T). For image processing Amira software (version 4.1.1, Mercury Computers Systems, CA, USA) was used. Mean and standard deviation (SD) of the signal intensities within each VOI were measured for calculation of the threshold values. For the contralateral side (VOI_4) the applied segmentation threshold was: $<VOI_4> - 3 \times SD(VOI_4)$. For VOI_3 – i.e. core region with contrast – the applied segmentation threshold was calculated from the mean of VOI_4 adjusted for possible bias determined from the means $<VOI_1>$ and $<VOI_2>$. A % bias correction factor was calculated as the percentage of signal difference between the outer layers of the two bulbs (VOI_1 and VOI_2): $100 - (100 \times <VOI_1>/<VOI_2>)$. The threshold for VOI_3 was then set as: $(<VOI_4> - <VOI_4> \times \%/100)) - 3 \times SD(VOI_4)$. The number of voxels demonstrating signal intensity below the thresholds was quantified for VOI_3 and VOI_4. Finally, the quantified number in VOI_3 was subtracted from the quantified number in VOI_4 as a correction for false positive hypointense voxels. Minimum intensity projections (mIPs) were created from a subsample of sagittal slices, covering the region where the RMS enters the OB (VOI_3), of the *ex vivo* 3D images acquired at 8 weeks PI.

Results Figure 2A shows the quantification of the number of voxels representing contrast spots at 3, 5 and 8 weeks for the four different injection setups. Only for the 10 µl MPIO injection and the 1.5 µl MPIOs-PLL injection, an accumulation of contrast spots in the olfactory bulb over time was observed. The number of pixels was around zero for the 1.5 µl MPIO injection and the 1.5 µl MPIOs-FUG injection at the three time points. On the mIPs of the 10 µl MPIO injection (Fig.4B) and the 1.5 µl MPIOs-PLL injection (Fig. 4C), the route of the RMS is marked as a clearly discernible dark dotted line and numerous dark spots are present in the olfactory bulb. This pattern of dark spots along the RMS and in the olfactory bulb is not visible on the mIPs of the 1.5 µl MPIO injection (Fig. 4D) and 1.5 µl MPIOs-FUG injection (Fig. 4E).

Figure 2



Conclusion and Discussion This method for contrast quantification provided evidence that contrast accumulation over time occurred in two out of the four *in situ* labeling strategies, indicating that these two setups were successful in labeling eNPC. It should be possible to use this method *in vivo* if the MRI resolution lies in the same range. This method could be useful to investigate altered SVZ neurogenesis in mouse models for different diseases with a longitudinal MRI study.

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References

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