Evaluation of LV and AAV vector systems for stable delivery of MRI reporter genes to the rodent brain

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INTRODUCTION

In vivo visualization of cells by utilizing viral vector-delivered MRI reporter genes for marking cell populations (e.g. endogenous neural stem cells by expression of ferritin[1]) will allow for stable cell labeling that is not lost upon cell division as compared to particle-based labeling methods. Lentiviral (LV) and adeno-associated (AAV) viral vector systems have proven very useful for stable, long term gene expression in mouse and rat brain[2,3]. Potential limitations of those systems for visualization by MRI are often insufficiently addressed. One such potential limitation is unspecific contrast by the vector itself. Here, we aim to evaluate the LV and AAV vector systems for their suitability to overexpress MRI reporter genes in mouse brain with regard to their background contrast on MRI.

METHODS

3 µl of LV-eGFP, AAV-eGFP-T2A-fLuc and PBS were stereotactically injected in the striatum of C57BL/6 mice (n=5). MRI images were acquired on a 9.4T small animal scanner (Bruker Biospin) using a linear resonator (transmit) in combination with a dedicated mouse head coil (receive). T_2- and T_2* maps (10 TE increments, 156 x 156 µm in plane resolution, 0.8 mm slice thickness) and 3D T2*-weighted images (FLASH, TR=100 ms, TE=12 ms, flip angle 30°. 59 µm isotropic resolution) were acquired from 24 hours to up to 5 months post injection. After the last time point, animals were sacrificed, *ex vivo* MRI of the excised brain were acquired (also a high resolution 3D T_2*-weighted FLASH with an isotropic resolution of 32 µm), and (immuno-) histochemical stainings were performed.

RESULTS

At 1day, 1 week and 1 month (shown in a) post injection, LV injection without MRI reporter genes causes clearly hypointense contrast at T2* MR at the site of injection that persists up to 5 months post injection, whereas a sham injection with PBS shows little to no contrast at the injection tract for all animals (n=10). The hypointense contrast does not correlate with transgene expression (see eGFP staining in c) but seems to correlate with the presence of Fe^{3+} (as revealed by DAB-enhanced Prussian blue staining, d) and accumulation of microglial cells (CD11b staining, e). Similarly, MRI 1 week and 1 month after AAV injection result in similar MRI findings as for the LV injection (n=5).

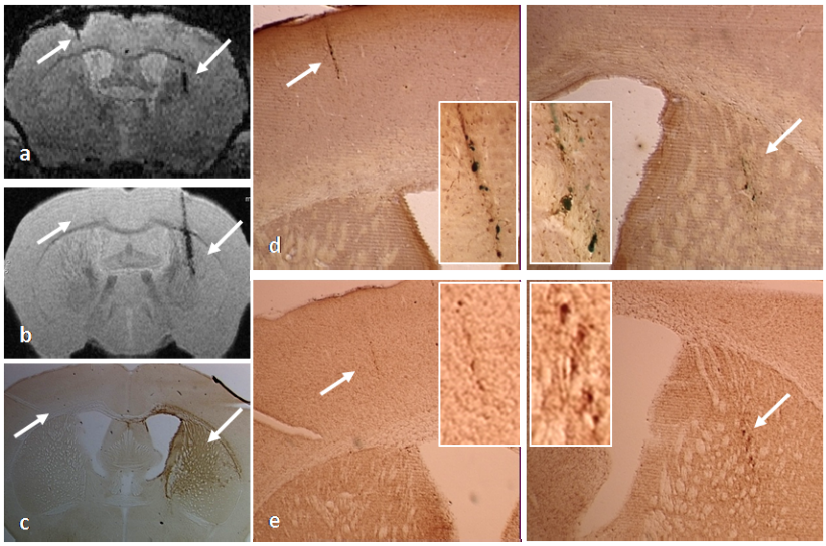

Figure 1: 3D T2*-weighted MRI after one months (a) *in vivo* and (b) of the excised brain. (c) eGFP staining, (d) iron staining and (e) CD11b staining of PBS (left) and lentiviral vector injection (right). The inset shows a magnification of the area designated by the arrow.

CONCLUSIONS

Viral vector injection itself results in a hypointense contrast at the site of injection on T2*-weighted MRI that cannot be explained by mechanical damage and/or bleeding inherent to the injection alone but seems to correlate with the presence of Fe^{3+} and microglial cells at the site of injection. These results challenge the signal-to-noise properties of any putative MRI reporter gene to be detectable above this background signal. When evaluating MR reporter genes (e.g. ferritin), care should be taken to use the appropriate viral vector control.

REFERENCES

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