Quantification of 3D T2*-weighted MR images allows evaluation of different viral vectors for stable MR reporter gene expression in the rodent brain

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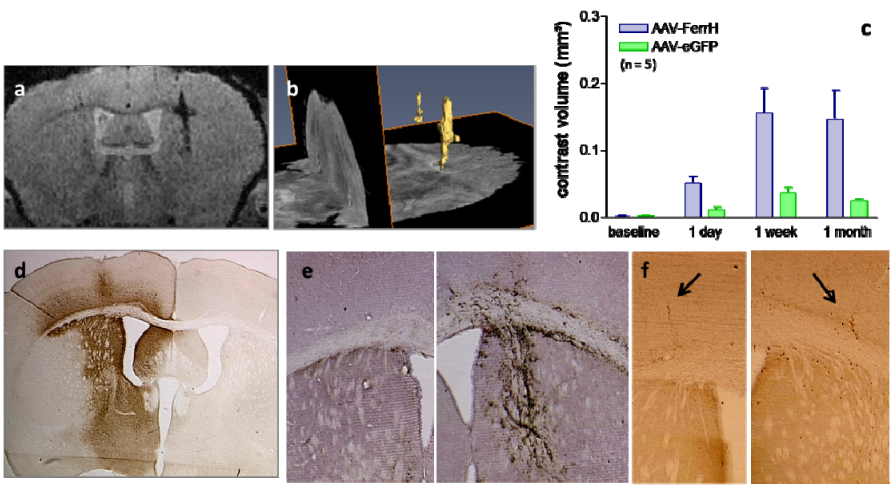
INTRODUCTION: For delivery of MRI reporter genes (e.g. ferritin) to the rodent brain, lentiviral (LV) and adeno-associated viral (AAV) vectors are the preferred viral vector systems when aiming for stable, long-term labelling and *in vivo* visualization of cells. However, their potential limitations for MRI like the generation of unspecific contrast by the vector injection and host response are often insufficiently addressed. Quantification of hypointense MR contrast using the parametric T_2-maps is often constrained by their low resolution. We developed an image processing pipeline for normalization and quantification of high-resolution 3D T_2*-weighted MR images. This pipeline allowed accurate and objective comparison of the potential of LV and AAV to be used as MRI reporter gene expressing vectors in the rodent brain with regard to their background contrast, both in immunodeficient and immunocompetent animals. **METHODS**: LV/AAV encoding MRI reporter (ferritin: LV/AAV-FerrH), different batches of control vectors (LV/AAV-eGFP) or PBS were stereotactically injected in the striatum of C57BL/6 and NOD-SCID mice (n = 5 per condition). 3D T_2*-w MR images (FLASH, TE=15ms, TR=150ms, flip angle 30°, isotropic resolution 59 µm), T_2 (MSME) and T_2* maps were acquired at different time points (t=1, 7, 14, 30, 60, 90 days) post injection (p.i.) on a 9.4T small animal scanner (Bruker Biospin, Ettlingen, Germany). After the last time point, animals were sacrificed, followed by *ex vivo* MRI of the excised brain (3D T_2*w, isotropic resolution 32 µm) and (immuno-) histochemical stainings. Our image analysis pipeline tackles the spatial normalization of these 3D T_2*-w MR time series images, using our maximization of mutual information (MMI)[1] algorithm. Its 12-parameter affine transformation addresses the image alignment to intra/inter animal, to anatomical template, as well as for *ex vivo* to *in vivo* registration, accounting for brain shrinkage. Also, we have implemented the information minimization method[2], for MR intensity inhomogeneity correction approach, without which image registration and quantification derails. Finally, the inter scan intensity variation[3] in time-series MR images were eliminated by correcting the intensity profile of the reference region. The bias corrected and normalized (spatial & intensity) 3D T_2*w images were then automatically quantified for the hypointense vector contrast by thresholding the volume-of-interest's defined in stereotactic coordinates. **RESULTS**: Injection in rodent brain of LV without MRI reporter (2 different batches of LV-eGFP) resulted in persisting hypointense contrast at the injection site on T_2*-weighted MRI. This contrast coincided with the presence of Fe^{3+} and microglia/ macrophages and could not be explained by bleeding or damage inherent to the injection (as evaluated from a PBS injection). In immunodeficient mice, the background contrast of LV was significantly reduced. For AAV, the background contrast was reduced by at least 50% as compared to LV. AAV-mediated ferritin overexpression resulted in more pronounced hypointense contrast as compared to AAV-control vector injection in immunocompetent mice (fig. a-c). This contrast coincides with the presence of Fe(III), without obvious microglia/macrophage infiltration (fig. e-f). **CONCLUSIONS**: LV vector injection itself results in hypointense contrast at the site of injection on T_2*w MRI, which is at least partially explained by an immune response. In contrast to LV, AAV vector injection results in very low background contrast levels and AAV-mediated MRI reporter gene overexpression results in significant contrast-to-background on MRI. Our image analysis pipeline permits the comparison of 3D T_2*w MR signal intensities over time points or specimen across different experimental groups.



Evaluation of MRI contrast mediated by AAV-FerrH. (A) Representative slice of 3D T_2*w *in vivo* MR images 1 month p.i. of a mouse injected with AAV-FerrH and AAV-eGFP in the right and left striatum, resp. (B) corresponding left and right contrast volume surfaces, delineated using image processing software on normalized T_2*w 3D MR dataset (1 month p.i.). (C) Graphic representation of contrast volume at different time points p.i. (D) immunohistochemistry (IHC) for eGFP showing a large area of transgene expression upon AAV-transduction (1.6x). (E) Prussian blue staining showing pronounced presence of iron at the site of AAV-FerrH injection (right) in contrast to the control AAV-eGFP injection site (left). (F) IHC using CD11b antibody; the arrows are indicating very few microglia/macrophages at the left and right injection tracts (4x).

1. F. Maes, A. Collignon, D. Vandermeulen, G. Marchal, and P. Suetens, "Multimodality image registration by maximization of mutual information," *IEEE Trans. Med. Imaging*, 16:2, 187–98(1997).
2. B. Likar, M.A. Viergever, and F. Pernus, "Retrospective correction of mr intensity inhomogeneity by information minimization," *IEEE Trans. Med. Imaging*, 20:12, 1398–1410, (2001).
3. Dominik S. Meier and Charles R. G. Guttmann, "Timeseries analysis of mri intensity patterns in multiple sclerosis," *NeuroImage*, 20:2, 1193–1209,(2003).