Imaging neural stem cell populations in the developing mouse brain using magnetic resonance micro histology

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Introduction: Advanced methods that enable labelling of neural stem cells (NSC) and progenitor cells (NPC) are fundamental for monitoring brain development under normal and pathological conditions. Magnetic resonance (MR) histology is an emergent technique that utilises MR microscopy and active staining techniques to provide enhanced tissue contrast for anatomical characterisation of transgenic mice [1]. Conventionally, this approach employs contrast agents to reduce image acquisition time and increase signal-to-noise ratio. However, a recent study [2] has demonstrated that contrast agents may also be used to differentially enhance contrast in specific tissues in the adult mouse, suggesting that MR histology may be able to provide an array of staining options to highlight distinct cellular structures. In this study, the biodistributions and MR relaxation mechanisms of a range of contrast agents have been investigated in the mouse embryo. We identify previously undetected substructures and delineate regions of NSC and NPC within the intact embryo brain.

Methods: C57BL/6 embryos (E15.5) were dissected, bled out and fixed for 2 weeks in a 4% formaldehyde solution doped with either: i) 8mM Gd-DTPA (Magnevist, Bayer-Schering Pharma); ii) 0.12mM $MnCl_2$ (Sigma-Aldrich); or iii) 8mM Mn-DPDP (Teslascan, GE Healthcare), and then embedded in 1% agarose doped with the corresponding concentration of contrast agent. Imaging was performed on a Varian 9.4T VNMRS system with a 33mm volume coil (RAPID Biomedical GmbH). T_1 and T_2* mapping data was acquired for a single sagittal slice (0.5mm thickness, matrix size 256x256 and FOV 27x27mm^2) using an inversion recovery spin echo and standard 2D gradient echo sequence, respectively. T_1 and T_2* maps were created using an in-house MATLAB program for comparison. High-resolution MR microscopy was performed on the Mn-DPDP-stained embryos using a 3D gradient echo sequence (FOV 27x27x27mm^3, matrix size 512x512x512, 7 averages) with optimised scanning parameters (TR=20ms, TE=4ms, FA=46°, NSA=4) simulated for maximum contrast between the heart and chamber using an in-house MATLAB program with mean T_1 and T_2* values measured from the respective maps. The resulting embryo volume images were compared with Gd-DTPA-stained embryo volumes previously acquired [3] with scanning parameters (TR=20ms, TE=9ms, FA=60°, NSA=7) optimised in the same way. Additionally, they were compared with histological sections stained with haematoxylin and eosin (H&E), which is a marker of nuclei and cytoplasm (i.e. cell density), and Nestin, which is a marker of NSC and NPC.

Results and discussion: The T_1 maps for embryos fixed in: a) 8mM Mn-DPDP; b) 0.12mM $MnCl_2$; and c) 8mM Gd-DTPA (Figure 1), exhibit marked tissue-specific differential staining (e.g. brain/heart T_1 ratio is 1.79 for Mn-DPDP, 0.83 for $MnCl_2$ and 1.05 for Gd-DTPA). It is possible that the contrast agents evenly distribute throughout all the tissues and that either: i) the inherent tissue relaxation properties have a marked effect at these very short T_1s and T_2*s; or ii) the relaxation rate of the contrast agents vary between different tissue types. However, the most apparent reason for this effect is that the contrast agents preferentially distribute to certain tissue and cellular structures leading to a local change in relaxation and enhanced contrast. This is illustrated in Figure 2, which compares high-resolution 3D images of: a) Mn-DPDP-stained embryos; and b) Gd-DTPA-stained embryos. The red arrows highlight regions showing differential contrast enhancement observed in the: i) cerebellum and midbrain; ii) eye; iii) pituitary gland; and iv) cortex. Our findings indicate that there are possible uptake and/or tissue dependent affinity differences between Mn-DPDP and Gd-DTPA in the developing mouse embryo. In particular, Figure 3 demonstrates that Mn-DPDP accumulates in regions of densely packed cells and clearly highlights the NSC and NPC in the ventricular zone of the cortex, midbrain and cerebellum, which is confirmed by the H&E- and Nestin-stained histological sections. This suggests that Mn-DPDP reveals regions of NSC and NPC, the cytoarchitecture of which influences the site-specific distribution of these contrast agents.

Conclusion: Distinguishing brain tissue sub-structure in *ex vivo* mouse embryos may be achieved using specific MR contrast agents, demonstrating that MRI may be able to offer flexibility and target specificity for phenotyping studies. Further investigation of how bio-distribution and relaxation mechanism contribute to this phenomena will enable development of new contrast agents that possess enhanced selective staining properties. This methodology enables visualisation of neural stem and progenitor cell populations, which may allow greater sensitivity for phenotypic characterisation of mutant mouse models, by highlighting specific cellular structures for investigation of the developmental and disease processes.

References: [1] Johnson G.A. et al. *Radiology* 2002, 789-793; [2] Huang S. *et al. Neuroimage* 2009, 46:589-599; [3] Cleary J.O. *et al. NMR Biomed* 2009, 22:857-866; [4] Modo. M.M.J. and Bulte J.W.M. *Molecular and Cellular MR Imaging*. Boca Raton, FL: CRC Press, 2007.

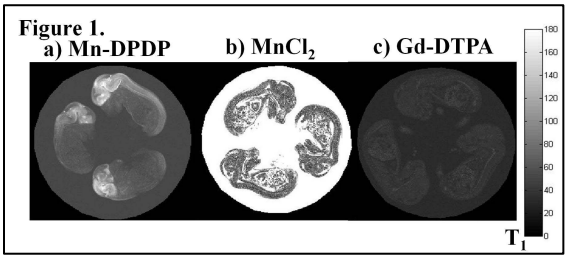

Figure 1. *T_1 maps for embryos stained with: a) 8 mM Mn-DPDP; b) 0.12 mM $MnCl_2$; and c) 8 mM Gd-DTPA.*

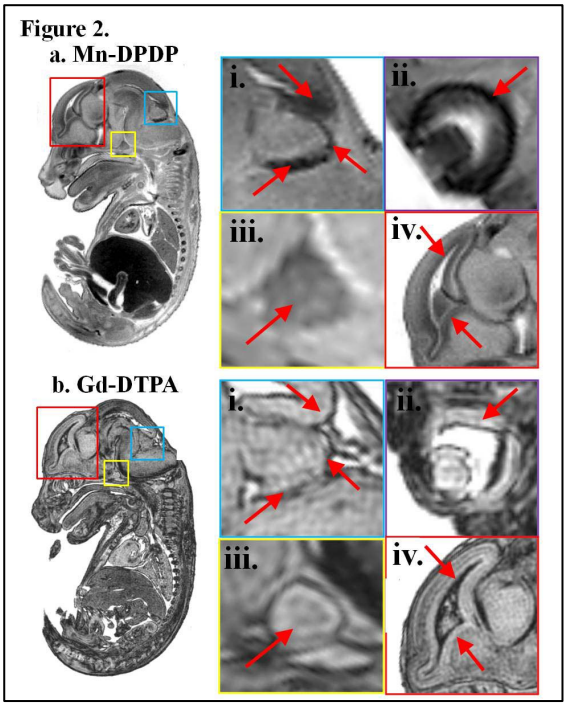

Figure 2. *3D gradient echo images for embryos fixed in a) Mn-DPDP and b) Gd-DTPA (resolution 54x54x54μm^3). Red arrows indicate differential contrast enhancement in the i) cerebellum and midbrain (blue); ii) eye (purple); iii) pituitary gland (yellow); and iv) cortex (red).*

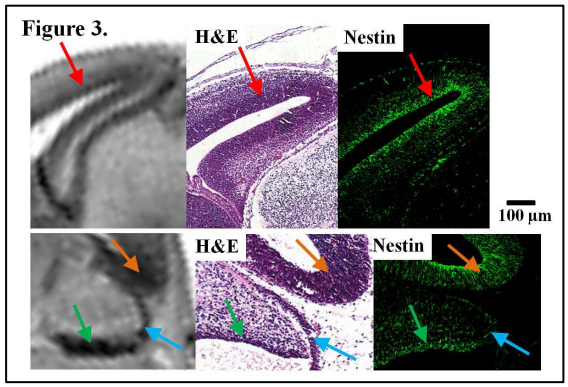

Figure 3*.MR images and histological sections stained with H&E and Nestin in the cortical ventricular zone (red), midbrain (orange), cerebellar ventricular zone (green) and external granular layer (blue).*