Mouse Embryo Phenotyping with Contrast-enhanced micro-Diffusion Tensor Imaging

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Introduction Mouse embryos are widely used as models for the study of human congenital abnormalities such as spina bifida, which is one of the most common birth defects. Morphological phenotyping of these embryos is still heavily reliant on histological sectioning: a destructive process that is time-consuming and operator dependent. Diffusion-tensor imaging (DTI) is a powerful technique that can explore tissue structure non-invasively, providing microstructural information such as the direction of tissue fibres. DTI techniques are particularly useful where traditional anatomical MR contrast is limited, such as in the developing foetal brain[1]. In the mouse embryo, DTI can characterise brain development[1] as well as examine phenotype in mutant animals[2]. However, these studies have imaged only the brain in mid-gestation embryos, with a modest number of diffusion directions (typically 6) and long acquisition times (~10 hours). Gadolinium chelates, such as Gd-DTPA, are commonly used in the fixation of embryos for MR microscopy, to reduce T1 and boost SNR[3]. While the use of Gd-DTPA is common in anatomical embryo imaging, its use has been limited in embryo DTI studies[4]. We have developed a contrast-enhanced technique for whole-body DTI of mid-gestation embryos, comparing embryo preparation, pulse sequences and resolution. Subsequently, we have applied our methodology to investigate the phenotype of the splotch mouse model of spina bifida, which carries a mutation in the gene Pax3[5] (a transcription factor implicated in the condition).

Methods 15.5 dpc embryos (C57Bl/6 background), were dissected from the mother and fixed for 2 weeks in a solution of 4% formal-PBS doped with 2mM Gd-DTPA. They were then moved to a solution of PBS+2mM Gd-DTPA for at least 2 weeks. Imaging was performed on a Varian VNMRS system using a 26mm volume coil (RAPID Biomedical GmbH) and 3D spin-echo (3D-SE) and fast-spin-echo (3D-FSE) diffusion sequences (diffusion parameters: G=50G/cm, Δ=3.5ms, Δ=8ms, 42 directions+6 b0 images), with parameters chosen to maintain sample temperature during the acquisition: SE: TR=500ms, TE=15.3ms, b=1550s/mm2; FSE: TR=500ms, TE1=14.75ms, ESP=5.75ms, ETL=4, b=1660s/mm2. Voxel size: 100x100x100µm3 and 75x75x75µm3 isotropic. 3D-SE T1 and T2 map data were also acquired. Sample temperature was 19±1°C. Images were corrected for movement by global registration[6]. Direction encoded colour (DEC) maps were visualised in MedINRIA (Asclepios Project); FA value calculation and tractography was performed in MRtrix[7].

Results After rehydration in PBS+Gd-DTPA, mean whole-body T2 increased from 14 to 25ms, giving improved SNR in the subsequent diffusion images (typically 39 vs. 22, 100µm SE data). DEC maps and values were similar between 3D-SE and FSE datasets (resulting 75µm DEC maps from FSE data are shown in Fig.1). Subsequently we imaged wild-type and splotch mutant mice, identifying differences in brain structure, in addition to spinal cord phenotype (Fig.2).

Conclusion In this study, we have shown that whole-body µDTI is able to delineate a number of anatomical brain regions according to diffusion directionality. We have used 42 directions in this embryo study, achieving high-resolution (75µm) and a moderately high b-value (1660s/mm2) in a 19h scan. Although we have concentrated on achieving high quality diffusion data for tractography through the acquisition of 48 images per scan, resorting to the minimum of 7 would give a total scan time of ~2.75 hours per embryo, improving throughput. As the final part of this study, we have described the phenotype of the splotch model of spina bifida for the first time with MR, characterising changes in brain structure and their relation to spinal cord phenotype. We hope our methodology may enable wider adoption of DTI to embryo studies, which may offer new insights into new and existing congenital disease models.

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