Diffusion Tensor Tractography Aids in Anatomical Phenotyping

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Introduction – Diffusion Tensor Imaging (DTI) of fixed mouse brain is useful in examining development (1,2) and genetic differences between wild type and knockout mouse models (3). Tractography using DTI, allows a qualitative visualization of changes in the morphology of white matter (4) but has had limited use in mouse phenotyping. Therefore, the purpose of this study was to assess anatomical changes non-invasively in the white matter of a genetically altered mouse model with associated white matter abnormalities.

Methods – *Specimen Preparation* – Six fixed mice brains were examined (3 wild-type and 3 Intersectin 1 and 2 double knockouts (DKO)). The mice were anesthetized and perfused. Following perfusion, the heads were removed along with the skin, lower jaw, ears and the cartilaginous nose tip. The remaining skull structures containing the brain were place in 4% PFA and 2mM Prohance (a Gadolinium contrast agent) overnight and then transferred to PBS, 0.02% sodium azide, and 2mM Prohance for at least 7 days prior to the MRI acquisition. *MRI Acquisition* – A multi-channel 7.0 Tesla MRI scanner (Varian Inc., Palo Alto, CA) with a 6-cm inner bore diameter insert gradient set (max gradient strength 100 G/cm) was used to acquire anatomical images of brains within skulls. Three custom-built solenoid coils were used to image three brains in parallel. A 3-D diffusion weighted fast spin-echo sequence was used with an echo train length of 6, a TR of 325 ms, first TE of 30 ms, and a TE of 6 ms for the remaining 5 echos, ten averages, field-of-view 14 x 14 x 25 mm^3 and a matrix size of 120, 120, 214 yielding an image with 0.117 mm isotropic voxels. One b0 image (with minimal diffusion weighting) and 6 high b-value images were acquired at a b-value of 1956 s/mm^2 in six different directions [(1,1,0),(1,0,1),(0,1,1),(-1,1,0),(-1,0,1),(0,1,-1)] for (G_x,G_y,G_z). Total imaging time was ~15.5 hours. Fractional Anisotropy (FA) maps were created and fibre tractography was performed on the images using DTI studio (DTIstudio software, Jiang and Mori, Johns Hopkins University, MD).

Results and Discussion – The Intersectin DKO is known, from histology, to have an abnormal corpus callosum (Sengar et al. unpublished), which was confirmed in the FA maps (Figure 1); furthermore the fornix and the anterior commisure also had abnormalities on the FA maps, which had not been seen on histology. To further asses these changes DTI tractoraphy of the white matter fiber bundles of those structures was performed. Fiber tracking was initiated in bilateral regions of interest for all three structures and was terminated if the FA dropped below 0.35 or the tract turning angle was larger than 70º. Figure 1 shows the resulting tractography in the wild type and the Intersectin DKO. The corpus callosum in the Intersectin DKO does not connect between hemispheres, seen in the FA map (yellow arrows) and using tractography. Reduced FA values were found in all three structures which were hypothesized to be caused by a smaller number of axonal projections.

Conclusions – DTI tractography allows for the detection of major neural fibre connectivity differences between mutant and wild type mice, which provides a non-invasive assessment for qualitative phenotyping of genetic disease models.

References – 1) Mori et al. *MRM* 46:18-23 (2001), 2) Baloch et al. *Cerebral Cortex* Epub (2008), 3) Wang et al. J of Neurocience 26:355-364 (2006), 4) Basser et al. MRM 44:625-632 (2000).

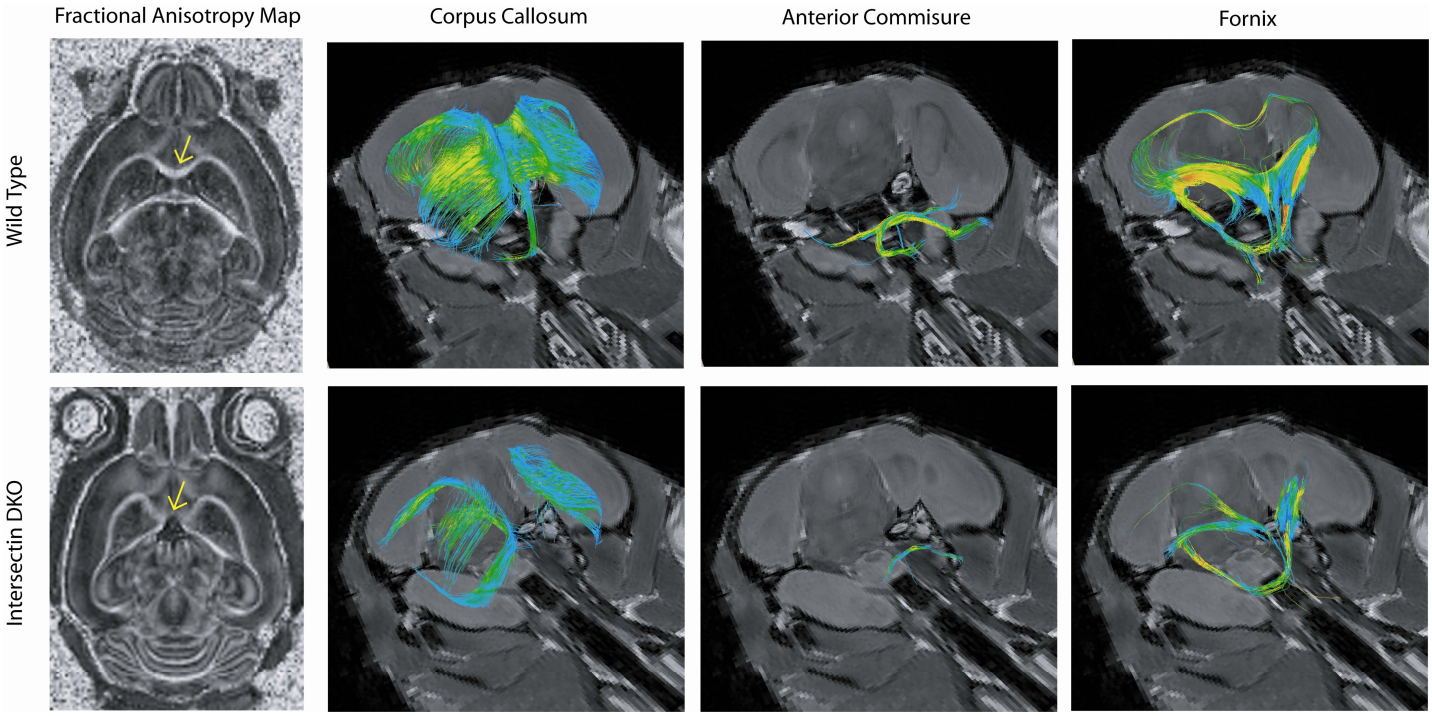

Figure 1 – Fractional Anisotropy maps as well as Diffusion Tensor fibre tactography of corpus callosum, anterior commisure, and fornix bundle of the wild type and Intersectin DKO mice.