Neocortex Organization and Connectivity in Fetal Human Brains Revealed by Diffusion Tractography and Histology

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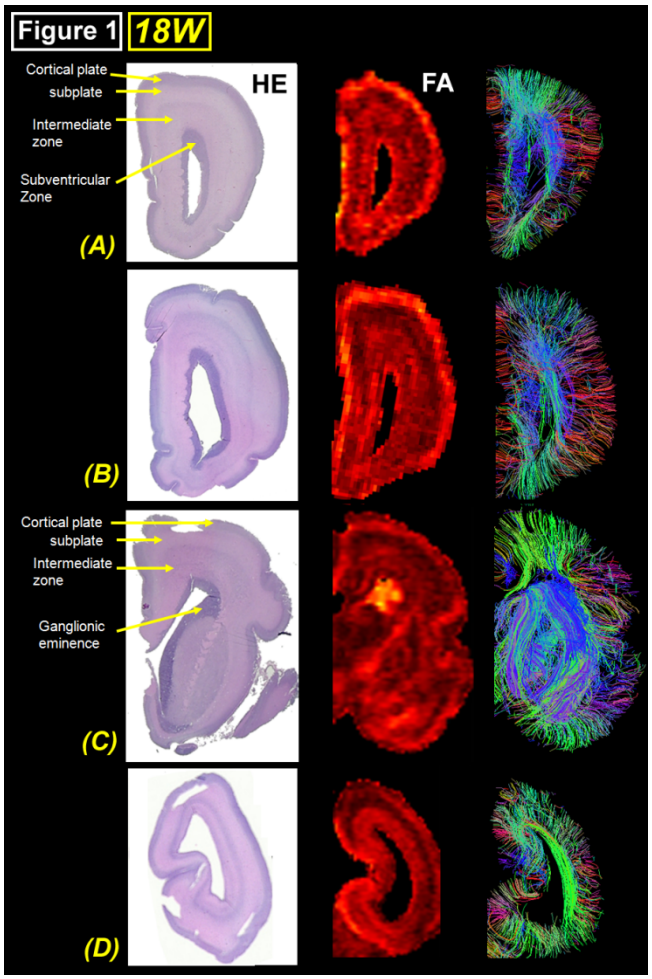
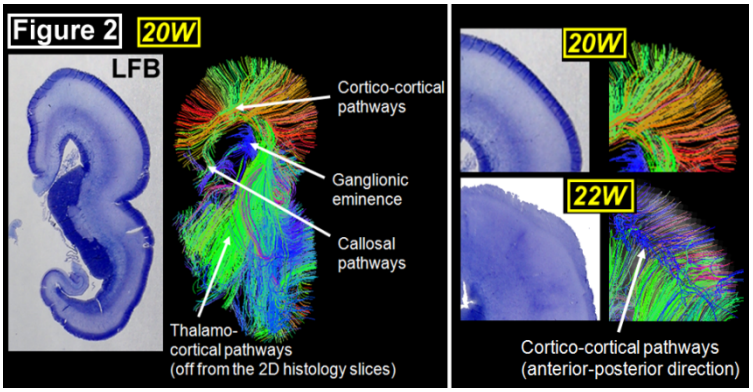
Introduction: During the late fetal period and early preterm period, the most prominent transient layer is the subplate (SP) zone, located between the immature cortex (cortical plate; CP) and the immature white matter (intermediate zone; IZ), which contains awaiting thalamo-cortical afferent fibers that cross one another **[1-3]**. The SP is especially thick in humans which develops around prenatal W13 and gradually disappears after W32–34 **[1, 3]** and contains complex crossing pathways. Resolving accurate pathways running through the unmyelinated subcortical areas is critical to image the entire length of fiber pathways in the developing brain. High-angular resolution diffusion imaging (HARDI) has been proposed as an alternative to diffusion tensor imaging (DTI) for improved resolution of crossing fiber pathways **[4]**, and is effective for delineating the structural changes that occur in developing fetal (preterm) brains **[5, 6]**. Here, we applied HARDI tractography to intact whole postmortem fetal human brains to explore the 3-dimensional evolution of the CP and SP into the mature cortical and immediately subcortical structure. We compared HARDI tractography, fractional anisotropy (FA), and histological measures (Hematoxilin and Eosin (HE) and Luxol Fast Blue (LFB)).



Methods: We used human fetal brain specimens of post-gestational week (W)18, W20, and W22 (two samples for each time point), using a 4.7T Bruker Biospec system. We performed a 3D diffusion-weighted spin-echo echo-planar imaging (EPI) sequence (61 measurements), with b = 8,000, small/large delta = 12.0/24.2 ms, TR/TE 1000/40 ms, spatial resolution 415 x 500 x 550 µm. Diffusion Toolkit and TrackVis were used for reconstructing and visualizing tractography. The color-coding of fibers is based on a standard RGB code, applied to the vector between the end-points of each fiber. After scanning, brains were sections at 10 µm, and stained by HE and LFB.

Results: ***At 18W***, the CP is dominated by a highly radial organization in the whole brain (Fig. 1 A-D, correspond to anterior to posterior brain slices) that appears to result from the persistent radial organization of neuronal columns along with radial glial pathways. At 18W, the outer SP is also highly radial, probably due to radially oriented SP neurons in addition to the emerging efferent/afferent axonal pathways. The deeper SP zone contained multiple crossing interhemispheric and intrahemispheric pathways, which resulted in low FA. In some brain region (e.g. an occipital region, Fig. 1 D), coherent cortico-cortical pathways were already observed in the IZ, but not in the whole brain at this stage.



These results agree with the known decrease in interhemispheric corpus callosum connections between visual areas with maturation and with the increase in ipsilateral corticocortical connections. ***At 20W***, myelin stains (LFB) were observed only at the border between the SP and IZ (**Fig.2 right panel**). Increasing types of pathways were running in the IZ. ***At 22W***, cortico-cortical pathways funning in the anterior-posterior direction were emerging at the border between the SP and IZ (**Fig. 2 left panel**). **Conclusion:** Our results show the usefulness of HARDI tractography in fixed pathological specimens, irrespective of the degree of myelination, for providing information on developing cortical structure and connectivity.

References: **[1]** Kostovic and Rakic, 1990; **[2]** Allendoerfer and Shatz, 1994; **[3]** Ulfig et al., 2000 **[4]** Tuch et al. 2003. Neuron, 40, 885-895. **[5]** Takahashi et al. 2010a. Neuroimage 49, 1231-1240. **[6]** Takahashi et al. 2010b. Cerebral Cortex (published online, DOI: 10.1093/cercor/bhq084).