Development of Cerebellar White Matter and Cortical Connections in Humans

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Introduction: The developmental time-course of the cerebellum is unique compared to the cerebrum **[1]**, and the cerebellum plays crucial roles not only in motor functions but also in higher cognitive functions in humans **[2]**. However, our understanding of the human cerebellum development has not advanced at the same level as our understanding of the cerebral development, because it is especially difficult to image 3-dimensional cerebellar connectivity using diffusion tractography due to the following reasons: 1) there are many narrow folia and therefore detecting tractography pathways in one folium are easily contaminated with a neighboring folium, and 2) there are many crossing axonal pathways in the cerebellum. High-angular resolution diffusion imaging (HARDI) has been proposed as an alternative to diffusion tensor imaging (DTI) for improved resolution of crossing fiber pathways **[3]**, and is effective for delineating the structural changes that occur in developing fetal brains **[4, 5]**. Here, we applied HARDI tractography to intact postmortem fetal cerebellums to explore the 3-dimensional development of cerebellum pathways.

Figure 1

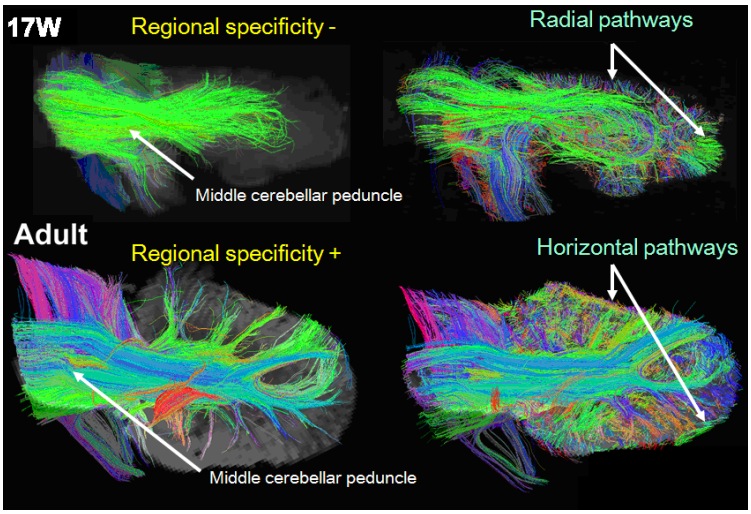
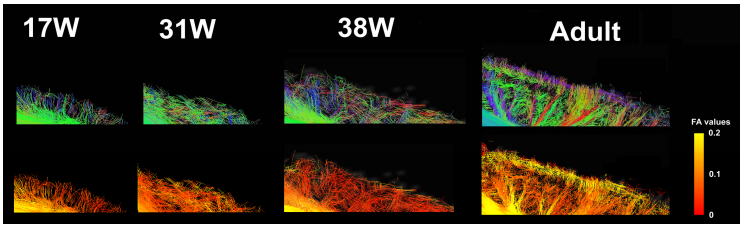


Figure 2



Methods: We used human fetal cerebellum specimens of post-gestational week (W)18, W22, W31, W38, as well as adult cerebellum specimens (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), TR/TE 1000/40 ms, with b = 8,000, small/large delta = 12.0/24.2 ms, spatial resolution 320 x 380 x 380 µm for W18-22, 425 x 425 x 500 µm for W31 and W38, and 600 x 730 x 760 µm for adult. The color-coding of fibers is based on a standard RGB code (Blue: dorsal-ventral, Red: right-left, Green: anterior-posterior).

Results:

Tractography at W18-W22:

Although main tracts of the cerebellar peduncles (superior, middle, inferior cerebellar peduncles, corticospinal tract and the medial lemniscus) was already developed, there was not obvious regional specificity at these stages (**Fig. 1 upper left**). The cerebellar cortex contained abundant radial pathways (**Fig. 1 upper right, Fig. 2**) but not horizontal pathways observed in later stages.

Tractography at W31- W38:

Horizontal pathways were emerging in the cerebellar cortex increasing their densities (**Fig. 3**).

Tractography in the adult:

Cerebellar peduncles were clearly imaged with regional specificity (**Fig. 1**). The upper half of the middle cerebellar peduncle at the level of pons projected to lower cerebellar hemisphere (blue in **Fig. 2 lower left**), and the lower half of the middle cerebellar peduncle at the level of pons projected to upper cerebellar hemisphere (green in **Fig. 2 lower left**), which was not clear at 18W (**Fig. 2 upper left**). Horizontal pathways further increased the densities in the cerebellar cortex (**Fig. 2, 3**).

Figure 2. Pathways in the cerebellar cortex. The color-coding of fibers is based on a standard RGB code (Blue: dorsal-ventral, Red: right-left, Green: anterior-posterior).

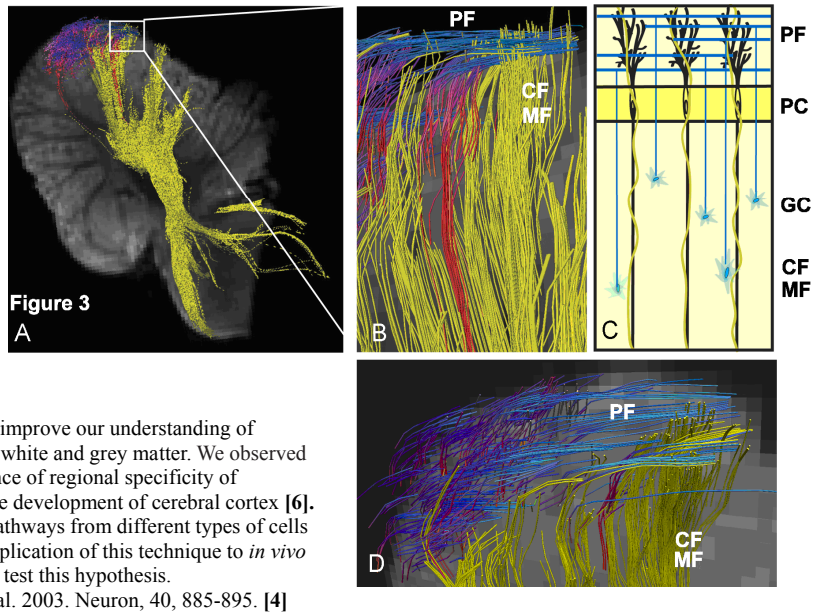


Figure 3. **A**. A low magnification view of the right hemisphere of an adult cerebellum in the axial plane is shown. The white box indicates a region of Crus I, which is magnified in **Fig. 3B**. **B**. Blue tractography pathways, consistent with parallel fibers, are observed running in a parallel direction to the folia. Yellow pathways are observed running perpendicular to these blue pathways. The orientation and direction of these tracks are suggestive of climbing fibers (CF), mossy fibers (MF), and axons of Purkinje cells (PC). This pattern of tractography pathways were observed in many regions of the cerebellum.
C. This schematic shows the anatomical relationships of the cells in the cerebellar cortex. Note the parallel fiber (PF) axons from granule cells (GC) running in the molecular layer of the cerebellum. These axons synapse with the terminal regions of the PC. The longitudinally oriented CF extends from the inferior olive nucleus, and synapses onto the Purkinje cell's dendritic tree. The MF from the cerebral cortex synapses onto the PF. **D**. Another example of this tractography pathway pattern is shown from a different region of the cerebellum.

Conclusion: Our results show the usefulness of HARDI tractography to improve our understanding of developing and adult cerebellar neural circuitry and connectivity in both white and grey matter. We observed regression of radial organization in the cerebellar cortex and the emergence of regional specificity of cerebellar peduncles that were similar to our previous observations on the development of cerebral cortex **[6].** In particular, our results suggest that we may be able to resolve axonal pathways from different types of cells within the cerebellar cortex, which is potentially critical for the future application of this technique to *in vivo* imaging. Future immunohistochemical correlation studies are planned to test this hypothesis.

References: **[1]** Volpe, 2008; **[2]** Schmahmann et al., 2006; **[3]** Tuch et al. 2003. Neuron, 40, 885-895. **[4]** Takahashi et al. 2010a. Neuroimage 49, 1231-1240. **[5]** Takahashi et al. 2011. Cerebral Cortex 21; 200-211. **[6]** Takahashi et al. 2011b, doi: 10.1093/cercor/bhr126.