Development of Cerebellar Connectivity in Human Fetal Brains Revealed by High Angular Resolution Diffusion Tractography

Emi Takahashi[1], Emiko Hayashi[1], Rebecca D Folkerth[2], Jeremy D Schmahmann[3], and Patricia E Grant[1]

[1]*Boston Children's Hospital, Harvard Medical School, Boston, MA, United States,* [2]*Brigham and Women's Hospital, Boston, MA, United States,* [3]*Massachusetts General Hospital, Boston, MA, United States*

TARGET AUDIENCE: Neuroradiologists and neuroscientists interested in brain development.

PURPOSE: High angular resolution diffusion imaging (HARDI) tractography has provided insight into major white matter pathways and cortical development in the human fetal cerebrum. Our objective in this study was to further apply HARDI tracography to the developing human cerebellum ranging from fetal to toddler stages, to outline in broad strokes the 3-dimensional development of white matter and local gray matter organization in the cerebellum.

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, ∂/Δ= 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

***Improved tractography results using a size-optimized MR coil for the cerebellum*:** Signal-to-noise ratio and tractography outcomes significantly improved using a size-optimized RF coil for each sample (**Fig. 1**). The cerebellum with the cerebrum attached was scanned using a large coil for a whole brain (**Fig. 1**, upper row), which produced many short irregular non-anatomic tractography pathways that are inconsistent with out knowledge of cerebellar anatomy. With a size-optimized coil for the cerebellum, after dissecting the cerebellum from the specimen, longer and more coherent tractography pathways were identified (**Fig. 1**, lower row).

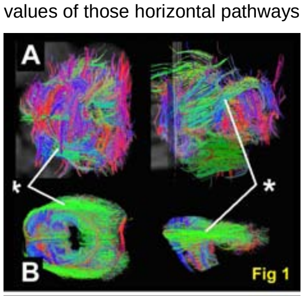
Cerebellar white matter pathways development: The corticospinal tracts and medial lemniscus were readily identified along with the superior, middle, inferior cerebellar peduncles at W17 (**Fig. 2A-C**). The tracks passing through the middle cerebellar peduncle did not reach the cerebellar cortex, but projected to broad areas in the cerebellar hemisphere, terminating deep to the cerebellar cortex (**Fig. 2D, E**). Pathways between deep cerebellar nuclei and the cerebellar cortex were not detected (**Fig. 2F**). In the adult cerebellum, the tracks passing through the middle cerebellar peduncle reached and projected to specific areas in the cerebellar cortex (**Fig. 2G, H**), and pathways between deep cerebellar nuclei and the cerebellar cortex were clearly observed (**Fig. 2I**).

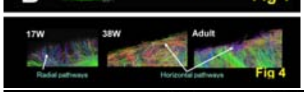
We then focused the analyses on the middle cerebellar peduncle (MCP) to study the emerging regional specificity of the tracts passing through this peduncle and their relationships to the cerebellar cortex. As the cerebellar folia developed, the course of the middle cerebellar peduncle became deeper to the cerebellar surface at 17W. Gradually the main body of the MCP tract took a deeper course from the surface of the cerebellum at and after 19W (**Fig. 3, arrowheads**). The middle cerebellar peduncle fibers did not reach the cerebellar cortex in W17 to W21. At W31, tracts passing through the middle cerebellar peduncle penetrating into the cerebellar folia were observed, and the number of these pathways entering the white matter laminae of the cerebellar cortical folia increased at W38 (**Fig. 3, asterisks**). Regional specificity of the fibers conveyed in the middle cerebellar peduncle also became more evident at W31-38.

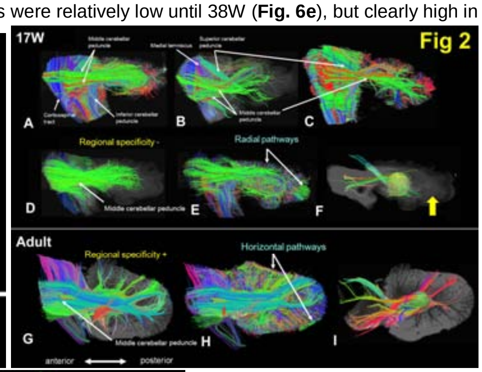
Cerebellar cortical pathways development: The cerebellar cortex contained dominant radial pathways at 17W in the sagittal plane (**Fig. 2E, Fig. 4**). Horizontal pathways emerged in the cerebellar cortex and increased in density at later stages (**Fig. 4**). Radial pathways were not detected in the axial planes at 17W (**Fig. 5**). Only a few cortical pathways were identified in axial planes at 17-21W. At 31-36W, cortical pathways gradually emerged mainly in a direction parallel to the pial surface, and only a few pathways were observed in the radial direction (**Fig. 5, white arrows**). At 38W, radial pathways in the cerebellar cortex increased their lengths coincident with increased thickness of the cerebellar cortex (**Fig. 5, white arrows** for example). We imaged both radial and horizontal cortical pathways crossing each other in the cerebellar cortex at this stage, but pathways running in the radial direction to the cerebellar surface were dominant.

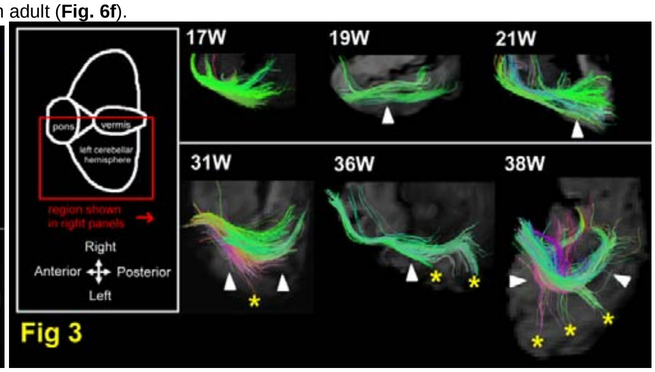
In the cerebellar vermis, only superficial pathways tangential to the surface running anterior to posterior were evident at 17W (green pathways) (**Fig. 5, green arrow**). At 21W, pathways parallel and tangential to the pial surface crossing one another across the vermis were observed (**Fig. 5, blue arrow**). Horizontal pathways gradually observed from W31, and at W36-38, the cerebellar vermis contained an increased number of distinct horizontal pathways (red pathways) (**Fig. 5, yellow arrows**).

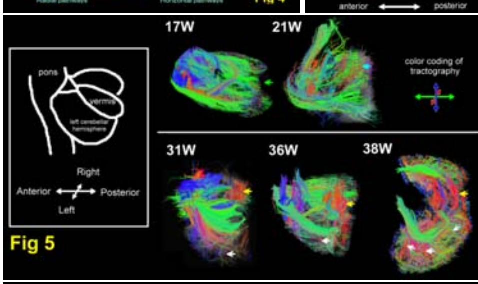
Changes in FA values during development: In the white matter, only deep white matter showed high FA values at W17-W31 (**Fig. 6a**). At W38, branching fiber pathways to the cerebellar cortex started to show high FA values (**Fig. 6b**). In the cerebellar cortex, abundant radial pathways with low FA values were detected at W17-W22 (**Fig. 6d**) but not horizontal pathways observed in later stages. Between W31 and W38, horizontal pathways were emerging in the cerebellar cortex, and increased in density (**Fig. 6e**). FA values of those horizontal pathways were relatively low until 38W (**Fig. 6e**), but clearly high in adult (**Fig. 6f**).

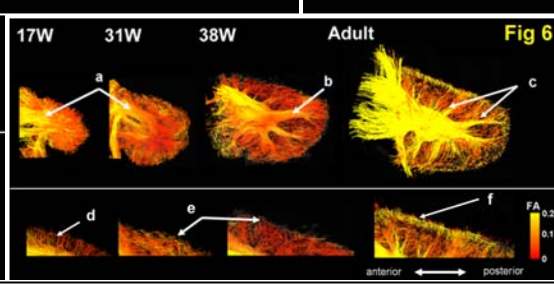












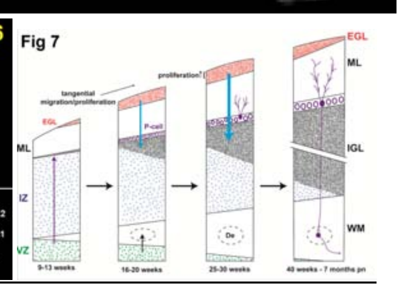


Figure 7: Summary scheme of developing cerebellar white matter and cortex pathways (redrawn from Sidman and Rakic, 1973). VZ: ventricular zone, IZ: intermediate zone, ML: molecular layer, EGL: external granular layer, WM: white matter, IGL: internal granule layer, De: dentate nucleus, P-cell: Purkinje cell.

DISCUSSION and CONCLUSION: Our results show the usefulness of HARDI tractography to image developing cerebellar connectivity. 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**.** Our results demonstrated the potential for HARDI tractography to improve our understanding of neuronal circuitry and connectivity in both white and gray matter in the developing cerebellum.