MR diffusion-based histology and micro-tractography reveal mesoscale features of the human cerebellum

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Purpose: Although a detailed cytoarchitectural description of the human brain by histology is long established, a comprehensive description of its connections ranging from major white matter pathways to small short-range fascicles of a few hundred axons remains elusive[1]. Diffusion imaging provides a non-invasive three-dimensional description of the microstructural organization of biological tissues and is one of the fastest growing neuroimaging techniques for investigating normal anatomy, mental health and neurological disorders in the living human brain. However, two major limitations of in-vivo diffusion imaging are the lack of histological validation and its intrinsic low spatial resolutions that limits our visualization to only the largest white matter pathways[1,2]. In this preliminary study, we show that high-resolution MR diffusion imaging applied to a fixed ex-vivo human cerebellum can be used to characterize the cytoarchitectural organization[3] of the cerebellum and, at the same time, offer a seamless integration of the cerebellar connections from macroscale to microscale.

Methods: The cerebellum of a 11 years old female was made available from the Clinical Neuropathology Department at King's College Hospital under the hospital post-mortem consent for medical and genetic research. The brain was extracted 45 hours after death from a pilocytic astrocytoma of the spinal cord and immersed in 10% neutral buffered formalin for 4 weeks. MRI images were acquired from the entire cerebellum and from smaller samples. High resolution MRI data was acquired using a 7T Agilent (Palo Alto, USA) MRI system. ***Diffusion MRI data*** was acquired using a Pulsed Gradient Spin Echo sequence (TR = 3600 ms, TE = 36 ms, G = 500 mT/m, δ = 4 ms, Δ = 15 ms, b-value $\approx$ 4200 s/mm^2, Averages = 170, 3 b0s and 30 DWI-directions, voxel size 100x100x200um). Total scan time was approx. 140 hrs. Data was first pre-processed correcting for eddy current distortions and for long-term movements of the sample using ExploreDTI. Diffusion tensor data was estimated using the full b-matrix and a non-linear least square approach. *Micro-tractography* was performed using an Euler-like integration with a step-size of 50 µm. Fractional Anisotropy (FA) thresholds were set to 0.07 to initiate and continue tracking. A lower FA threshold compared to in-vivo tractography was required due to the low diffusivity and anisotropy of the fixed sample. Angular threshold was set to 45º. After cutting histological sections (50µm) on a freezing microtome, ***Immunohistochemistry analysis***, was performed using specific primary antibodies to detect myelin, neurons, Purkinje cells, and Bergmann glia cells. Sections were visualised on a Zeiss Axioplan.

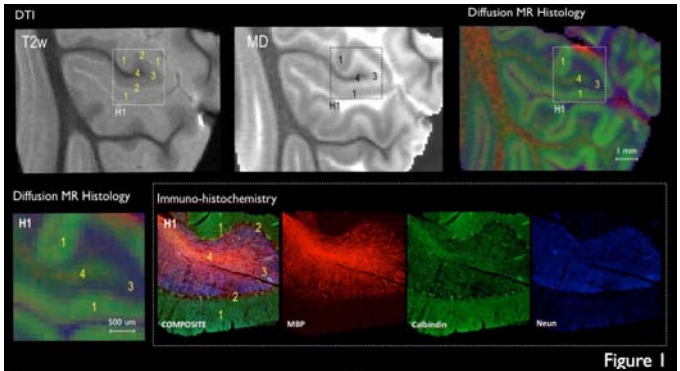

Figure 1

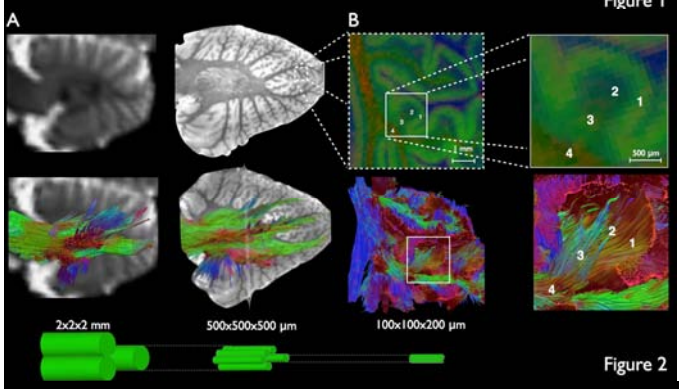

Figure 2

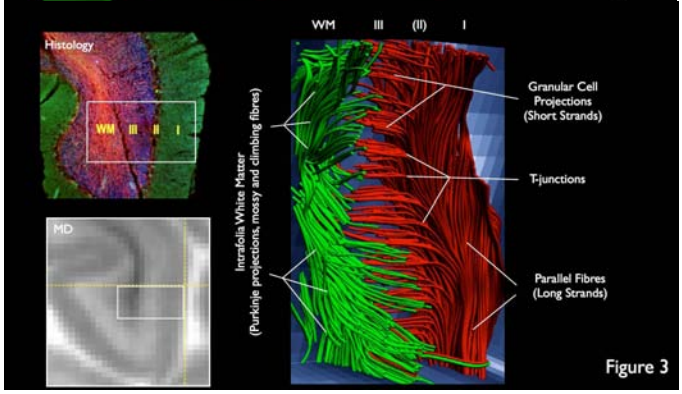

Figure 3

Results: *Figure 1*: At a spatial resolution of 100 µm, diffusion maps reveal the main cytoarchitectural features of the cerebellar cortex. White matter (4), granular layer (3), Purkinje cell layer (2) and molecular layer (1) can be discriminated by T2-weighted, mean diffusivity (MD) and Diffusion MR-histology maps. Diffusion MR-histology maps are obtained as pseudo-color visualization by combining together FA (red), mean-DWI (green) and MD (blue) maps to mimic immunohistochemistry results. Compared together immunohistochemistry and diffusion maps revealed a good matching by showing the same laminar organization across the entire imaged sample.

Figure 2A: As reference, on the left, a conventional clinical DTI tractography at a resolution of 2x2x2 mm (8 µl/voxel) reveals the major white matter tracts and macroscopic features of the cerebellum. On right with the fixed cerebellum acquired at 500x500x500 µm (0.25 µl/voxel, 32 times smaller) it is possible to further focus on the cerebellum to reveal a far greater number of anatomical details and connections.

Figure 2B: At a resolution of 100x100x200 µm (2nl/voxel, 4000 times smaller than the clinical scan) it is possible to observe mesoscale connectivity features of cerebellar white matter and histological details of the cerebellum grey matter. White-matter (4) and intra-folia axonal projections (3), granular cell layer (2), and the molecular layer (1) are all identifiable within each cerebellar folium. In particular, the presence of a structural continuum of parallel orientations, inside the molecular layer, is anatomically consistent with the presence of the cerebellar parallel fibres running inside this layer.

Figure 3: At a deeper level, features of the cerebellar intra-cortical connectivity are revealed by micro-tractography in a portion of cerebellar folium and they provide a glimpse of the microstructural organization within individual layers. White matter (WM) trajectories propagate and disperse inside the granular layer (III) of the folium. At this interface, repeated short strands, likely to be representative of the anatomy of a small group of hundreds of granular cell axons running coherently within each voxel, project to the molecular layer (I) and merge with almost orthogonal and longer strands that consistently describe the dominating orientation of the parallel fibers inside this layer. Taking into account the limitations of tractography reconstructions based on DTI, these trajectories are consistent with the anatomical cerebellar system of granular cell axons, T-junctions and Parallel fibers[4].

Conclusions: Diffusion MR histology and micro-tractography represent two novel approaches for obtaining a three-dimensional descriptions of tissue cytoarchitecture and white matter organisation at a mesoscale level. These methods produce quantitative information that, coupled with high-resolution visualisation of small fibres, can fill the gap existing today between in-vivo macroscopic large-scale network mapping and microscopic histology analysis. Results in this study have been obtained first using DTI to provide a baseline of what achievable at this resolutions. Further acquisitions implementing more advanced diffusion models (e.g. HARDI[2], DSI[5]) are currently on-going. In conclusion, we believe that this approach can represent an essential step forward in understanding the connectivity of the human brain.

References: *[1] Mesulam M. NeuroImage. 2012; 62 (4): 2182–2189. [2] Jones D.K. Cortex. 2008; 44: 936-952. [3] Shepherd T.M. et al. Am J Neuroradiol. 2007; 28:958-964. [4]J Voogd & M Glickstein. Trends Neurosci. 1998. 21, 370–375. [5] Wedeen VJ et al. Magn Reson Med. 2005; 54(6):1377-1386.*