LONGITUDINAL ANALYSIS OF THE CORTEX USING DIFFUSION-MRI IN AN ANIMAL MODEL OF CORTICAL DYSPLASIA

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INTRODUCTION: Focal cortical dysplasia (FCD) is a malformation of cortical development that often leads to drug-resistant epilepsy and is characterized by cortical layer disruption and abnormal neurons (Blümcke et al., 2010; Najm et al., 2022). The temporal evolution of this type of malformation remains unclear and often goes undetected by conventional imaging due to their heterogeneity and subtlety (Spitzer et al., 2022). Here we present the use of diffusion-MRI as an alternative to assess the evolution of microstructural abnormalities in the cortex by using an animal model of cortical dysplasia. **OBJECTIVES:** Characterized the magnitude and temporality of the cito- and myelo- architecture changes in the cortex throughout advanced diffusion-MRI (dMRI) techniques and their histological validation. **METHODS:** Six Sprague-Dawley rats were injected with either carmustine (BCNU; n=3) (Benardete & Kriegstein, 2002) or saline solution (Control; n=3) at the embryonic day 15. The resulting pups (BCNU =16 | Control =16) were scanned *in vivo* at the 30, 60, 120 and 150 post-natal days with a preclinical 7T Bruker scanner. Here we acquired anatomical T2-weighted images (TR/TE: 4212.78/33 ms, spatial resolution of 0.117 x 0.117 x 1 mm^3) and diffusion-weighted images (TR/TE: 2000/22.86 ms, spatial resolution of 0.175 x 0.175 x 1 mm^3) with b values of 670, 1270 y 2010 s/mm^2, and 90 directions each. Along with fourteen b=0 s/mm^2. Our dMRI were preprocessed with denoising (Veraart et al., 2016), Gib's ringing (Kellner et al., 2016) and Eddy correction (Andersson et al., 2003). For our dMRI analysis, we applied a Laplacian potential field (Lerch et al., 2008) to create a 2D grid-lines system as a common anatomical descriptor. This coordinate system has fifty parallel lines spanning the cortical ribbon, each one with ten equidistant points. Here we fitted the diffusion tensor (DTI) (Basser et al., 1994) and a novel multi-tensor approach known as multi-resolution discrete-search (MRDS) (Coronado-Leija et al., 2017). MRDS can fit up to three independent tensors per voxel, each with its own eigenvalues and eigenvectors. We sampled derivatives metrics from DTI and MRDS and conducted a Linear Mixed Effect Model analysis at every vertex (points). Finally, to validate the cyto- and myelo architecture, we performed histological assessments using the primary antibodies MBP (1:200), NeuN (1:350) and Foxp2 (1:2000). To analyze the MBP staining, we calculated the structure tensor using the OrientationJ software (Püspöki et al., 2016). **RESULTS:** The longitudinal T2 images showed hippocampal atrophy and enlarged of the ventricles after the postnatal day 60. Our dMRI analysis did not reveal significant changes in the DTI metrics at any temporal point, while MRDS showed significant differences ($p<0.05$) between groups in the metrics FAperp, FApar and MDperp, particularly at 30 postnatal days. This dMRI results were supported by the lack of myelin and disarrangement of the fibers at the level of the motor cortex and the derivatives coherency maps from the structure tensor at 30 postnatal days. In addition, the cyto-architectural stains with NeuN demonstrated a blurring and undefined transition between the cortical layer VI-V and III-IV, along with non-well defined cortical columns at every time point. **CONCLUSION:** Our findings indicate that during the early stages of development (30 days), macrostructural alterations in FCD are exceedingly subtle and remain undetectable through conventional anatomical imaging. However, our use of dMRI has proven to be valuable tool for identifying microstructural abnormalities. While DTI did not reveal significant

changes in diffusivity, our MRDS approach successfully pinpointed cortical regions with abnormal microstructure at 30 postnatal days related to disarrangement myelin radial-tangential fibers and blurring columnar-layer architecture, as confirmed by histological analysis. These findings put forward the potential use and application of advance dMRI for the detection of human FCD.

Acknowledgements for technical assistance of Nydia Hernández-Ríos, Ericka de Los Ríos, Juan Ortiz-Retana, Gema Martínez and Leopoldo González Santos. Project funded by UNAM-DGAPA (IN2044720)