Characterizing Neuronal Morphological Development Using Diffusion Tensor Imaging

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Introduction: Herein, the possibility that morphological differentiation of neurons in the early-developing cerebral cortex can be monitored by diffusion tensor imaging (DTI) is investigated. Immediately after pyramidal neurons migrate from periventricular zones to the cortical plate, neuronal arbors consist of singular, radially oriented neurites. At this stage of development, water diffusion in the cerebral cortex is significantly anisotropic. As neurite arbors grow into a mature configuration, multiple collaterals form in directions ranging from perpendicular to parallel to the pial surface. The width of distribution of neurites therefore increases, and water anisotropy diminishes (1). Superimposed on the temporal decrease in cortical diffusion anisotropy are regional differences, which arise from a gradient in neurogenesis, and differences between primary and non-primary cortical areas (2). It is hypothesized here that variation in axonal/dendritic orientation distributions measured using Golgi staining procedures will correspond to DTI measurements performed on the same tissue according to a recently proposed quantitative model. Association of DTI measurements with specific morphological characteristics of neurons is expected to contribute toward utilization of DTI as a diagnostic technique for detecting neurodevelopmental disorders associated with improper cerebral cortical differentiation.

Methods: Post-mortem DTI measurements were performed on right hemispheres of three female ferrets (postnatal day(P) 13, P20, and P31). A Stejskal-Tanner multi-slice spin echo pulse sequence (0.15 mm^3 voxel size) was executed on a Bruker-Biospec 11.7 T MRI system. An icosahedral diffusion sampling scheme was used to acquire 25 b=2500 s/mm^2 images (and two b=0 images). Additional settings were δ = 12 ms, Δ = 21 ms, TR $\approx$ 10 s, TE = 42 ms, with 6 repetitions.

Hemispheres were subsequently cut axially on a cryostat in sections of 0.15 mm^3, and processed using the Rapid GolgiStain™ kit (FD NeuroTechnologies, Inc., Ellicott City, MD). Regions of interest (ROIs) within the Golgi stained data were imaged via light microscopy for qualitative comparison to DTI data. High-resolution 3D ROIs of confocal images were digitized using back-scattered light at wavelength 633 nm for quantitative comparisons. A 3D skeletonization algorithm was applied to the confocal ROIs, and dendrite orientations determined from contiguous segments of at least 10 voxels. Distributions of neurite orientations were characterized in terms of a 3 × 3 orientation matrix, **T** (3), and compared to the diffusion tensor, **D**, via the relation

$$0.5\nu(D_{\parallel}-D_{\perp})(\mathbf{T}-\mathrm{Tr}(\mathbf{T})/3) = \mathbf{D}-\mathrm{Tr}(\mathbf{D})/3 \qquad [1]$$

in which $D_{\parallel}$ and $D_{\perp}$ are water diffusion coefficients relative to the local neurite axis, and ν is the volume fraction of the neuropil (4).

Results: Figure 1 displays FA maps calculated from DTI measurements for P13 – P31 (scale bars = 1mm). In Figure 2 panels a – d show 1 mm × 1 mm regions of cerebral cortices from P13 and P20 brains. Neurons and associated processes exhibit radial orientations. As seen in panels 2e and 2f, this radial orientation is largely reduced by P31, though regional variability in width of the orientation distribution is apparent in comparisons between the two P31 panels. Throughout maturation, wider distributions of branch orientations are evident in primary areas (panels a, c and e, primary visual cortex) than in non-primary areas (panels b, d and f, frontal cortex). These regional patterns are also reflected in Figure 1 FA values.

In Figure 3, the top panel displays Golgi-stained tissue from a P31 brain with specific ROIs demarked. The middle panels are representations of the distribution of neurite orientations calculated from confocal data in regions A and B depicted in the top panel. The bottom panel is a graphical representation of the relationship between measured FA (Diffusion, Eq. 1 right side) and quantitative indices of neurite orientation distributions (Neurite orientation, Eq. 1 left side). Measurements from the ROIs depicted in the top panel were used to determine this relationship. Measured FA (via DTI) was found to significantly predict quantitative indices of neurite orientation distributions according to Eq. 1 ($F_{2,12}$ = 16.5, $p < 0.005$).

Conclusions: Abnormalities in morphological differentiation of cerebral cortical neurons are associated with several neurodevelopmental disorders (5). Although the likely dependence of diffusion anisotropy on immature pyramidal neuronal morphology has been hypothesized (1), direct comparisons have yet to be reported. Our findings confirm that temporal and regional changes in cortical diffusion anisotropy coincide with expansion of axonal/dendritic arbors. This correspondence suggests DTI measurements can in principle be extended to quantitatively characterize morphological development of the cerebral cortex *in vivo*.

References: 1. McKinstry RC et al. Cereb Cortex **12**:1237 (2002), 2. Kroenke CD et al. J Neurosci **27**:12506 (2007), 3. Fisher, NI et al. Statistical Analysis of Spherical Data, Cambridge University Press (1987), 4. Jespersen SN et al. NeuroImage **34**:1473 (2007), 5. Kaufmann, WE and Moser, HW Cereb Cortex **10**:981 (2000).

Fig. 1

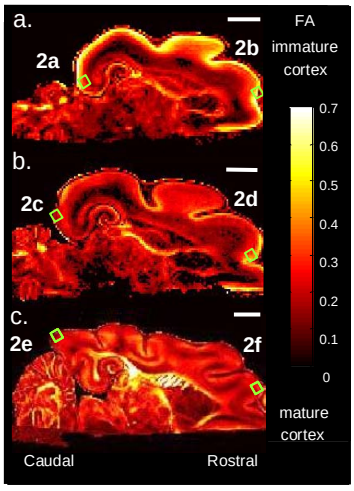

Fig. 2

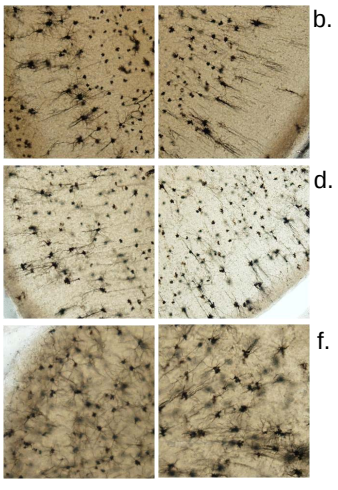

Fig. 3