Diffusion Spectrum Tractography and Histology: Developing Connectivity in the Cat Brain

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Introduction Transient patterns of cerebral laminar organization and exuberant connections are prominent features of immature brains. For example, the transient subplate (SP), located just below the immature cortex (cortical plate; CP), is crucial for the formation of normal neuronal circuits. There are three major concerns relevant to the use of conventional DTI tractography for studies of the immature brain. First, a single voxel can contain more than one fiber orientation, as in cases when multiple fibers cross one another within a voxel. The SP is known to contain abundant crossing pathways during development. Second, the immature brain is less myelinated than the adult brain, which leads to lower overall FA values in white matter where tractography tends to be erroneously terminated. Third, the spatial resolution of most *in vivo* DTI studies is insufficient to fully study cortical organizational changes. At birth, the cerebral cortex is approximately 1 mm thick and therefore the standard 2 mm x 2 mm x 2 mm voxel for DTI cannot probe cortical structure in detail. Using high-resolution diffusion spectrum imaging (DSI) tractography, we successfully imaged the 3-dimensional structure of the cortical and subcortical pathways in P0 (newborn), P35 (pediatric), and P100 (adult) cats and compared these tractography findings to histology.

Methods We performed scans on the brains of two newborn cats (postnatal day 0; P0), two kittens (P35) and two young adult cats (P70 and P100). After the cats were euthanized, their brains were perfused with phosphate buffer saline (PBS) solution followed by 4% paraformaldehyde, removed from the cranium and fixed in 4% paraformaldehyde containing 1 mM gadolinium (Gd-DTPA) MRI contrast agent for 1 week to reduce the T1 relaxation time while ensuring that enough T2 -weighted signal remained. For MR image acquisition, the brains were placed in the Fomblin solution (Fomblin Profludropolyether; Ausimont, Thorofare, NJ). We used two Bruker Biospec MR systems: a 9.4 T scanner for the two P0 specimens and a 4.7 T system for the other specimens. The pulse sequence used for image acquisition was a 3D diffusion-weighted spin-echo echo-planar imaging (EPI) sequence, TR/TE 1000/40 ms, with an imaging matrix of 112 x 112 x 128 pixels for P0 brains, 96 x 112 x 128 pixels for P35 brains, and 96 x 96 x 128 pixels for P70 and P100 brains. Spatial resolution was 300 x 300 x 300 μm for the newborn kittens, 420 x 420 x 420 μm for the juvenile kittens, and 550 x 550 x 600 μm for the young adult cats. We performed diffusion spectrum encoding as previously described (Wedeen et al., 2005). Briefly, we acquired 515 diffusion-weighted measurements, corresponding to a cubic lattice in Q-space contained within the interior of a ball of maximum radius b_{max} = 40k, with small delta = 12.0 ms, large delta = 24.2 ms. The total acquisition time was 18.5 hours for each experiment. Diffusion Toolkit and TrackVis (http://trackvis.org) were used for reconstructing and visualizing tractography pathways.

Figure 1

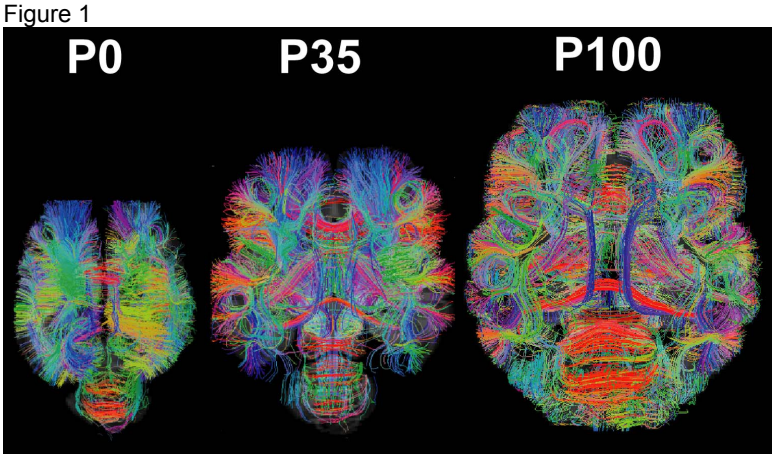


Figure 2

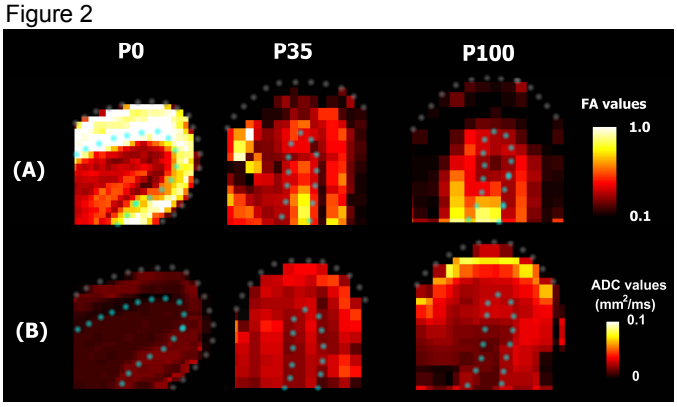
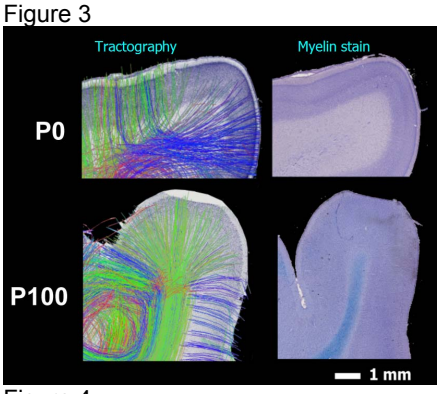


Figure 3

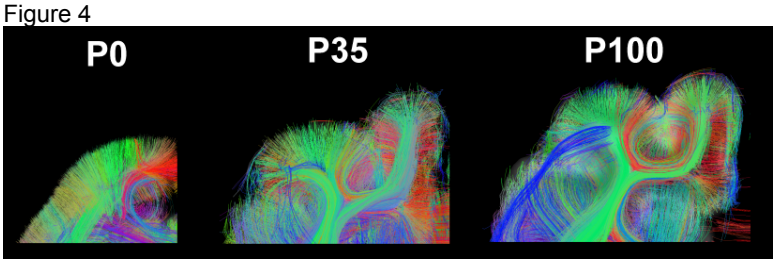


Results: Whole brain DSI tractography fibers were detected at all ages as the gyral structure developed (Fig. 1). We compared fractional anisotropy (FA) maps (Fig. 2A), apparent diffusion coefficients (ADC) maps (Fig. 2B), diffusion tractography pathways, Thionin stainings, and Luxol Fast Blue counterstained with Nissle stainings (Fig. 3 middle and right).

On DSI tractography, the CP and external SP had a highly radial organization at P0 that persisted but was less pronounced at P35 and P100 (Fig.2). This organization appears to correlate best with the radial neuronal organization that has the highest cellular density at P0, while entering and exiting connections may also play a role (Fig. 3 middle). On DSI tractography, abundant crossing pathways were detected in the deep SP and immature white matter at P0, although there was almost no myelin stained at this stage. At later stages, the white matter had increased directional coherence compared to the P0 cat (Fig. 3 middle), which well correlated with myelin stain at P100 (Fig. 3 right).

In the P35 and P100 brains, regions rich in crossing pathways as in the inner SP at P0 were not uniformly observed but became more restricted to specific gyri containing long association pathways (Fig 4). At P35 and P100, fibers in gyri also became more compartmentalized with subcortical U-fibers at the periphery of the gyrus and longer-range projection pathways exiting more centrally.

Figure 4



In some regions perpindicular to the projecting pathways, long association fibers were also identified. This regional varation become more prominent from P35 to P100.

These results suggest that DSI tractography successfully depicted the developmental change of organization of gray matter and white matter, and will contribute to our understanding of early brain development, and also show the potential of DSI in fixed pathological specimens at any stage of myelination to provide information on developing organization and connectivity.

Reference: Wedeen et al., MRM 2005