High throughput three-dimensional imaging of myelin fibers in the whole mouse brain

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Introduction. Injury to the white matter (WM) leads to severe functional loss in many neurological diseases such as stroke and multiple sclerosis. In-vivo neuroimaging techniques such as diffusion tensor imaging are becoming important tools for the diagnosis of WM abnormalities. However, these tools still present limitations and there is a crucial need for whole-brain ex-vivo complementary approaches to overcome them [1]. We propose a novel three dimensional (3D) imaging method to map the myelin fibers in the whole mouse brain with high throughput using in-line phase contrast X-ray tomography.

Methods. Twelve C57Bl6 mice were included in the present study (stroke: N=3, focal demyelination: N=2, healthy&sham: N=7). The impact of fixation was examined in 5 of the healthy brains fixed either by paraformaldehyde (PFA) 4% or ethanol (range [25%-96%]). All subsequent brains were fixed with ethanol 96%. Imaging was performed on beamline ID19 at ESRF at 19keV as described in [2]. An indirect detection-based detector with a LuAg scintillator, standard microscope optics and a 2048x2048 pixel CCD camera was positioned 1-m from the sample to obtain phase contrast. The whole-brain data set was acquired at an isotropic pixel size of 7.5-µm. Acquisition time was 14 minutes per brain. 3D images were reconstructed by Filtered Backprojection after phase retrieval based on [3].

Results. In contrast to PFA fixation, ethanol >50% led to hyperintense WM fibers, allowing accurate 3D representation of main fiber tracts (Fig 1). Our imaging approach revealed the complexity of fiber orientations and intrication, and distinguished individual axons in the fiber bundle (see st in Fig 1A). Focal WM lesions were clearly depicted both at the macroscopic (Fig 1: CPu and int demyelination, plain arrow; other lesions, arrow heads) and microscopic (Fig 1: st demyelination, thin arrow) levels.

Conclusion. The proposed set-up permits brain-wide studies of fiber tracts and of their structural changes in diseases, using conventional animal models, minimal sample preparation, and with the potential to scan 30 brains in an 8-hours shift.

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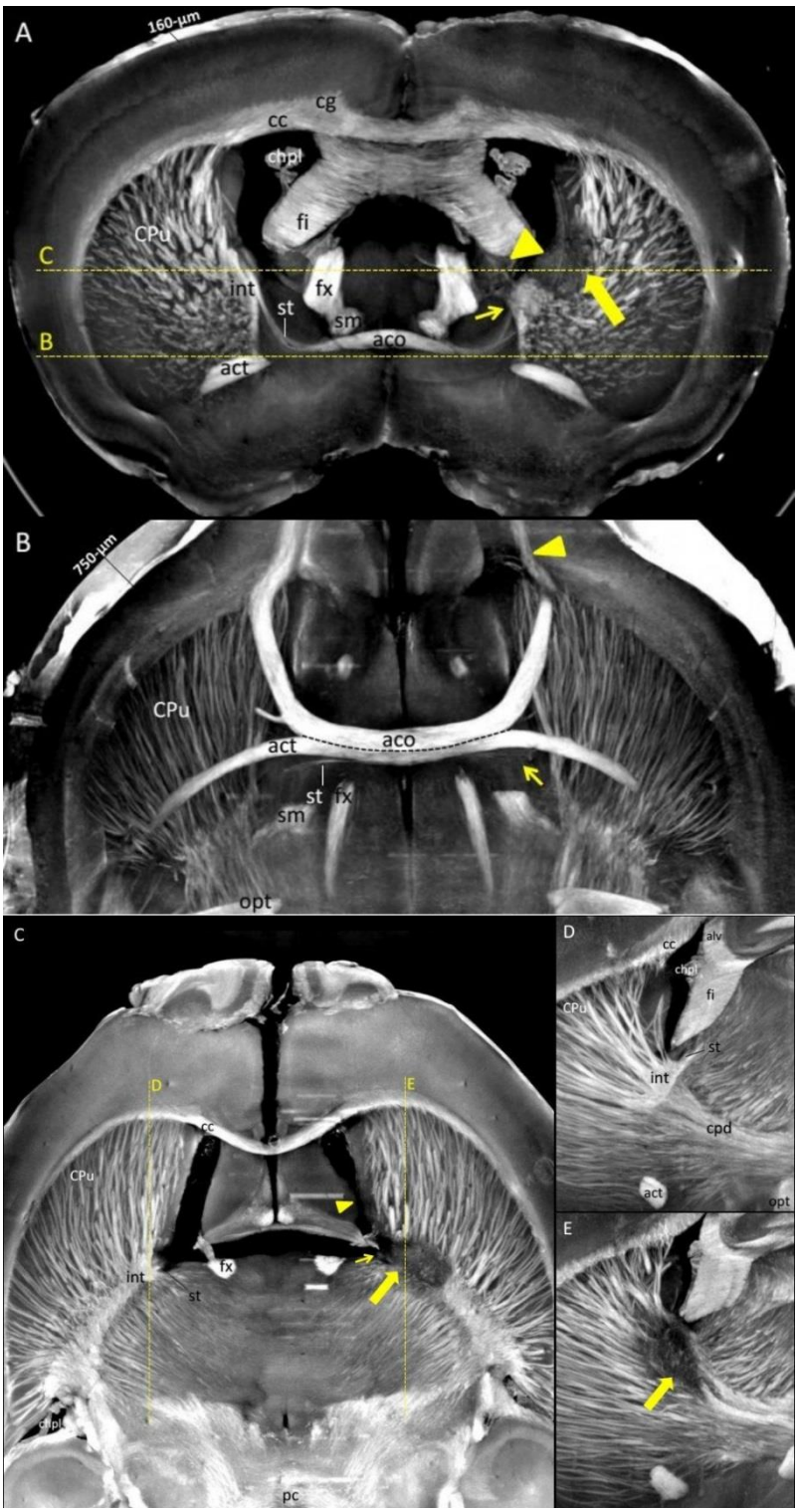

Figure 1: MIP in an animal model of demyelination: axial (A), coronal (B), contralateral sagittal (D) and ipsilateral sagittal (E) views. Focal demyelination of caudate putamen is clearly seen (plain arrow in A, C et E). Other focal lesions are shown in the ipsilateral (left) side with an arrow head: disorganization of the bed nuclei of the st (A), demyelination of the aco (B) and lateral ventricle collapse (C). Demyelination of stria terminalis is also detected despite the small size of this fiber tract (thin arrow in A, B and C).
Abbreviations: aco anterior commissure, olfactory limb; act anterior commissure, temporal limb; alv alveus; cc corpus callosum; cg cingulum; chpl choroid plexus; cpd cerebral peduncle; CPu caudate putamen; df dorsal fornix; fi fimbria; fr fasciculus retroflexus; fx columns of the fornix; int internal capsule; mtt mammillothalmic tract; opt optical tract; pc posterior commissure; sm stria medularis; st stria terminalis..

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