Visualization of Mouse Barrel Cortex using *Ex-vivo* Track Density Imaging

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Introduction: The arrangement of whisker follicles on the rodent's snout is highly conserved and can be mapped precisely onto the primary somatosensory or barrel cortex. The pathway between the whiskers and the cortex comprises connections between neurons in the trigeminal nuclei of the brainstem, the ventral posterior medial (VPM) and posterior medial (POM) thalamic nuclei and the barrel cortex. The functional organization, development and plasticity of the barrel cortex are of great interest in neuroscience[1].

The low-resolution structure of barrel cortex can be indirectly visualized using *in vivo* BOLD FMRI with whisker electrical stimulation, which reveals signal change in the micro and macro-vasculatures surrounding each barrel column[2]. Reliable high-resolution imaging of the barrel cortex has been difficult to achieve using Magnevist enhanced *ex-vivo* samples, possibly because of variability in binding of Magnevist to individual barrels and the grey matter tissue separating the barrels.

Recently, we described a new method to achieve super-resolution using diffusion MRI for *ex-vivo* mouse brain[3]. The super-resolution track-density imaging (TDI) technique is able to significantly increase the spatial resolution of the reconstructed images beyond the acquired MRI resolution. In essence, this is achieved by generating a very high number of streamlines, using whole brain probabilistic fiber tracking, followed by counting their number on a high-resolution grid[4]. In the directional encoded color short tracks (DECst) TDI variant, the directionality of the tracks within the grid were encoded by RGB colors and incorporated into the TDI map. DECst TDI maps showed fine mouse brain structures and correlated well with histological data [3]. ***In this study, we apply DECst TDI to produce high-resolution images of the barrel cortex.***

Methods: Four 12-week old adult C57BL6 mice were anaesthetized and perfused with 4% paraformaldehyde containing 0.5% Magnevist. The brains were removed from the skull and placed in Fomblin. MRI data were acquired on a 16.4T scanner (Bruker Biospin) using previously published protocols[5]. The acquisition consisted of (1) 3D Diffusion-Weighted Spin-Echo sequence with the parameters TE/TR= 22.8/400ms, 0.1mm isotropic resolution, 30 uniformly distributed DW directions, b=5000s/mm^2 with an acquisition time ~32hrs (n=2) or with 1.5 zero-fill encoding acceleration in the phase and slice dimensions to shorten the acquisition time to ~15h (n=2); (2) 3D gradient echo (GE) sequence with TR/TE/FA= 50ms/12ms/30°, 82 KHz spectral bandwidth, 4 excitations with an acquisition time of ~2.5h to produce T_1/T_2*-weighted images at 30µm isotropic resolution.
Separately, 10 brains were imaged using various Magnevist concentrations and incubation times during the trials to visualize the barrels using 3D GE. In addition, this study also compares the barrel visualization using the data already acquired (n=18) used to build the Australian Mouse Brain Mapping Consortium (AMBMC) 15µm resolution C57 BL6 atlas.

DECst-TDI maps were calculated as previously described[3]. Whole-brain fibre-tracking was performed using the program MRtrix[6] with constrained spherical deconvolution (CSD)[7] to model multiple fibre orientations (maximum harmonic order, *lmax*=6), and probabilistic fibre-tracking was performed using 2nd order integration over fibre orientation distributions (iFOD2)[8]. The following parameters were used: 0.1mm step-size, maximum angle between steps = 45° and 3 FOD samples/step, any track with length < 0.4 mm was discarded and the termination criteria were: exit the brain or when the FOD amplitude was < 0.01. To avoid intensity saturation in the TDI maps, the maximum track length was set to 1.0 mm^3. Sixty-five million tracks were generated for each mouse data set, and the DECst TDI maps were generated using a 20µm isotropic grid.

Results: The barrel structure could not be visualized on the individual brains that make up the AMBMC adult C57/BL6 mice (n=18). The barrel structures were still invisible after averaging the brains to create the atlas, which can be due to normal inter-brain variability. This trend is contrary to the other grey matter structures such as the thalamic nuclei, which become enhanced after averaging (Abstract 1610 "15µm average mouse models in Waxholm space from 16.4T 30µm images"). In contrast, DEC-stTDI permitted the reliable detection of the barrel cortex in all brains. Their precise pattern can be distinguished, as the individual barrels appear hypointense due to the low number of streamlines in these areas compared to the surrounding grey matter. The spatial arrangement of barrels visible on DEC-stTDI corresponds well with histological sections.

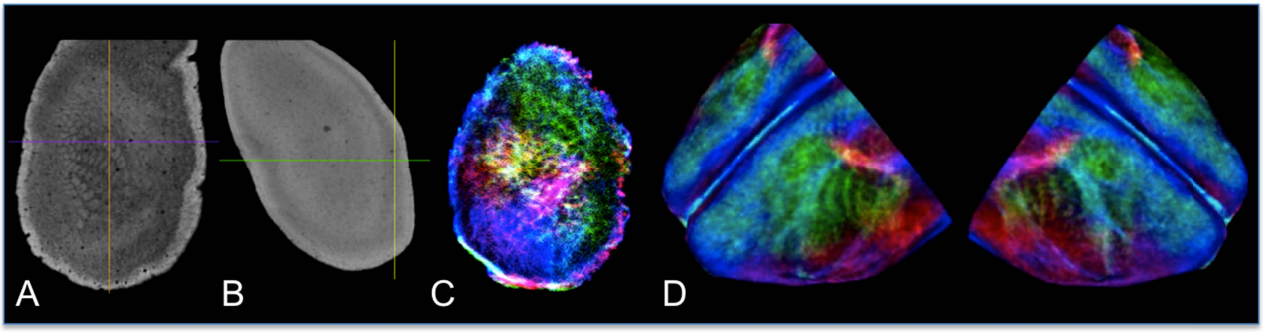

Figure 1. DECst-TDI can visualize the structure of barrel cortex consistently compared to Magnevist-enhanced 3D-Gradient Echo imaging. (A) A 3D Gradient Echo image showing a tangential slice for the barrel cortex. While this particular example showed an excellent contrast, only few brain samples (<10%, n=10) showed clear delineation of the barrel structures. (B) Average of 18 adult C57/BL6 mice. (C) DECst-TDI map registered and the plane selected at the same angle to the average brain. The barrel structures can be seen in all of the samples processed with DECst-TDI (n=4). (D) Volume rendering of the DECst-TDI map after eroding the surface of the cortex to layer IV.

Conclusion: The barrel cortex structure can now be visualized clearly and reliably using the DEC-stTDI technique. This technique is a significant advance in our ability to map structural and functional changes in the somatosensory barrel cortex caused by genetic or acquired lesions.

References: (1) Petersen CC *Neuron* 2007;56:339. (2) Yu X, et al. doi:10.1016/j.neuroimage.2011.08.001. (3) Calamante F, et al. *Neuroimage* 2012;59:286. (4) Calamante F, et al. *Neuroimage* 2010;53:1233. (5) Moldrich RX, et al. *NeuroImage* 2010;51:1027. (6) *MRtrix*, http://www.brain.org.au/software/ (7) Tournier JD, et al. *NeuroImage* 2007;35:1459. (8) Tournier JD, et al. *Proc. ISMRM*, 2010;18:1670.