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Visualization of mouse barrel cortex using ex-vivo track density imaging

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ABSTRACT

We describe the visualization of the barrel cortex of the primary somatosensory area (S1) of ex vivo adult mouse brain with short-tracks track density imaging (stTDI). stTDI produced much higher definition of barrel structures than conventional fractional anisotropy (FA), directionally-encoded color FA maps, spin-echo T_1- and T_2-weighted imaging and gradient echo T_1/T_2*-weighted imaging. 3D high angular resolution diffusion imaging (HARDI) data were acquired at 48 micron isotropic resolution for a $(3\ mm)^3$ block of cortex containing the barrel field and reconstructed using stTDI at 10 micron isotropic resolution. HARDI data were also acquired at 100 micron isotropic resolution to image the whole brain and reconstructed using stTDI at 20 micron isotropic resolution. The 10 micron resolution stTDI maps showed exceptionally clear delineation of barrel structures. Individual barrels could also be distinguished in the 20 micron stTDI maps but the septa separating the individual barrels appeared thicker compared to the 10 micron maps, indicating that the ability of stTDI to produce high quality structural delineation is dependent upon acquisition resolution. Close homology was observed between the barrel structure delineated using stTDI and reconstructed histological data from the same samples. stTDI also detects barrel deletions in the posterior medial barrel sub-field in mice with infraorbital nerve cuts. The results demonstrate that stTDI is a novel imaging technique that enables three-dimensional characterization of complex structures such as the barrels in S1 and provides an important complementary non-invasive imaging tool for studying synaptic connectivity, development and plasticity of the sensory system.

Introduction

The arrangement of whisker follicles on the rodent snout is highly conserved and can be mapped precisely onto the cytoarchitectural barrel fields located in the primary somatosensory (S1) cortex (Woolsey and Van der Loos, 1970). Whiskers actively move at high frequency and are thought to have a similar function to the human fingertips for sensing surface texture (Petersen, 2007). Functional and anatomical mapping studies have established that each whisker makes a preferential connection to a single barrel (Grinvald and Hildesheim, 2004; Simons and Woolsey, 1979; Welker, 1976). In rodents, barrels in the large barrel field, referred to as the posterior medial barrel subfield (PMBSF), are arranged topographically in 5 rows (A–E) with 5 arcs (1–5). The cross-sectional area of PMBSF is approximately 1 mm^2, which is 40% of the total barrel field area. In cross-section the principal axes of the largest barrels in the PMBSF (B1, C1) are approximately 170 and 380 μm. The stereotypical organization of the barrel field provides an important anatomical reference used to study synaptic connectivity, development, and plasticity of the sensory system (Daw et al., 2007; Petersen, 2007).

Currently, the barrel field is visualized using optical imaging methods involving immunohistological staining for choline acetyltransferase (ChAT), glucose transporter-2, the 5HTT serotonin transporter or cytochrome oxidase C (Gonzalo-Ruiz et al., 1995; Land and Simons, 1985; Voutsinos-Porche et al., 2003). While immunohistochemical methods produce clear barrel delineation, processing techniques involve sectioning or removal of the cortex from the whole brain followed by pressure flattening prior to vibratome sectioning. The loss of cortical curvature and tissue distortion not only renders three-dimensional volumetric reconstruction challenging, but also affected the accuracy measurements

Abbreviations: CSD, Constrained spherical deconvolution; DEC, Directionally encoded color; DTI, Diffusion tensor imaging; DWI, Diffusion-weighted imaging; FOD, Fiber orientation distribution; FA, Fractional anisotropy; HARDI, High angular-resolution diffusion-weighted imaging; SH, Spherical harmonics; stTDI, Short-tracks track density imaging; TDI, Track density imaging; VPM, Ventral posteromedial thalamic nucleus; PMBSF, Posterior medial barrel sub-field.

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of individual barrel column angles (Egger et al., 2012). More recently 3D auto-fluorescence optical imaging has been used (Gleave et al., 2012). Auto-fluorescence methods are non-destructive but involve dehydration of the sample in ethanol resulting in significant artifacts from inhomogeneous tissue shrinkage.

Magnetic resonance imaging (MRI) has also been employed to visualize the barrel cortex. Using in vivo blood-oxygenation level dependent functional MRI (BOLD fMRI), the low-resolution structure of the barrel cortex has been indirectly visualized during whisker electrical stimulation through signal changes in the microvasculature surrounding each barrel column (Yu et al., 2012). Ex-vivo MRI has been used to reveal brain microstructure (Dorr et al., 2008; Ma et al., 2005; Ullmann et al., 2012); however, observation of individual barrels in the barrel cortex has not been reported.

Recently, a new super-resolution track-density imaging (TDI) technique was developed to increase the spatial resolution of reconstructed images significantly beyond the acquired MRI resolution. The technique provides very high anatomical contrast based on anatomical connectivity (Calamante et al., 2010). It entails the application of constrained spherical deconvolution (CSD) to high angular resolution diffusion imaging (HARDI) data (Tournier et al., 2007) followed by the generation of a large number of streamlines using whole brain probabilistic fiber-tracking. The intensity of TDI maps corresponds to the number of streamlines that are present within each high-resolution grid element (the voxel of the super-resolution map). To increase the contrast in ex-vivo C57BL/6J mouse brain diffusion imaging, Calamante et al. (2012a) introduced TDI maps using short tracks instead of full-length streamlines. Short tracks TDI (stTDI) maps have the advantage of reducing saturation within structures with a high-density of long tracks (such as the corpus callosum and the internal capsule). Structures with shorter streamlines within the gray matter, such as the molecular layer of the hippocampus, are more clearly visualized with stTDI (Calamante et al., 2012a). In directional encoded color stTDI (DEC stTDI), the directionality of the tracks within the grid is also color-mapped using the standard red–green–blue convention.

In this study, we compared stTDI with conventional diffusion tensor analysis and T_1, T_2 and T_2^* weighted imaging methods to visualize the stereotypical architecture of the barrel cortex. Images were compared to histological data from the same animal by registering the MRI scans with 3D reconstructions of tissue sections in which the barrel field was visualized using virus-mediated fluorescence labeling. Finally, we tested the sensitivity of stTDI to detect changes in the barrel field resulting from infraorbital nerve cut.

Materials and methods

Sample preparation

Twelve-week old adult C57BL/6J mice were anesthetized and perfused with 4% paraformaldehyde containing 0.5 mM Magnevist®. The brains were removed from the skull and placed in PBS containing 0.5 mM Magnevist® for 4 days. The samples were imaged using perfluoroether Fomblin Y06/06 solution medium (Solvay Solexis, Italy). All mice were housed and handled in accordance with the Queensland Animal Care and Protection Act 2001 and the current NHMRC Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (UQ animal ethics approval CAI/375/10/NHMRC/NF and Florey Institute of Neuroscience and Mental Health AEC 10-090). MRI data were acquired on a 16.4 T scanner (Bruker BioSpin) using previously published protocols (Moldrich et al., 2010).

Ex-vivo MRI

Whole brain ex vivo MRI datasets were acquired using a 15 mm SAW volume coil (M2M Imaging, Australia). (1) HARDI data were acquired using a 3D diffusion-weighted spin-echo sequence with the parameters TE/TR = 22.8/400 ms, 100 μm isotropic resolution, 30 uniformly distributed DW directions, b = 5000 s/mm^2, δ/Δ = 2.5/12.5 ms, NEX = 1 with an acquisition time ~32 h (n = 7) or with 1.5 zero-fill Fourier encoding acceleration (partial FT) in the phase dimensions to shorten the acquisition time to ~15 h (n = 5). Fourier transformations were performed with the resolution of the k-space data being increased by 50% to 66 μm isotropic resolution to reduce the interpolation error in estimating the fiber orientation distribution (FOD) coefficient used in fiber-tracking (Tournier et al., 2012). (2) Conventional T_1/T_2^*-weighted anatomical images were acquired using a 3D GE sequence with TR/TE/FA = 50 ms/12 ms/30°, 82 kHz spectral bandwidth, and 4 excitations with an acquisition time of ~2.5 h to produce images at 30 μm isotropic resolution.

The images described above were compared with the 30 μm resolution 3D T_1/T_2^*-weighted GE data (n = 18) used to construct the average model for the Australian Mouse Brain Mapping Consortium C57BL/6J atlas (Johnson et al., 2010; Ullmann et al., 2012). Prior to scanning, these brains were incubated for 4 days in 0.5 mM Magnevist®. The average model was created by a recursive non-linear hierarchical fitting strategy and nonlinear transformation of the images in the Waxholm stereotaxic coordinate space (Johnson et al., 2010) and interpolated to create a model with 15 μm^3 isotropic voxels.

To optimize specimen preparation for barrel visualization using 3D GE, a further 10 adult mouse brains were also imaged at Magnevist® concentrations of 0.5, 1.0 and 2.5 mM with incubation times of 1 and 4 weeks using the imaging parameters described above. A set of sequences were additionally tested on whole brain ex-vivo samples to assess the potential of conventional MRI methods for barrel visualization: (1) 3D T_1/T_2^*-weighted GE at 30 μm isotropic resolution with TE = 20 ms, NEX = 12, acquisition time = 15.5 h and TE = 12 ms, NEX = 8, acquisition time = 13 h. (2) T_1-weighted 3D spin-echo (SE) at 50 μm isotropic resolution with TR/TE = 400/11 ms, NEX 1, acquisition time = 4 h. (3) T_2-weighted 3D SE both at 50 μm isotropic resolution with TR/TE = 1000/35 ms, NEX = 2, acquisition time = 13 h. (4) T_2-weighted 3D fast spin-echo (FSE) both at 50 μm isotropic resolution with TR/TE = 1000/35 ms, RARE acceleration factor 4, NEX 4, acquisition time = 3 h.

Ultra-high resolution cortical slab imaging

A (3 mm)^3 block of fixed adult brain tissue containing the barrel field in one hemisphere was cut to fit into a 5 mm SAW coil (M2M Imaging, Australia). The acquisition included: (1) HARDI dataset using 3D DWI-SE with the parameters TE/TR = 22.8/400 ms, 48 μm isotropic resolution, 30 uniformly distributed DW directions, δ/Δ = 2.5/12.5 ms, b = 5000 s/mm^2, and NEX = 2 with the acquisition time of ~22 h. (2) a HARDI dataset with identical parameters apart from 100 μm isotropic resolution, NEX = 1 and an acquisition time of ~5 h. The k-space resolutions were not increased prior to Fourier transform in these HARDI datasets. (3) 3D GE with the parameters TR/TE/FA = 50 ms/12 ms/30°, NEX = 4, and 10 μm isotropic resolution, with an acquisition time of ~2.5 h with 1.3 factor of zero-fill Fourier encoding accelerations in the phase-encoded dimensions.

Diffusion parametric image reconstructions and fiber-tracking

Diffusion tensor imaging (DTI) parametric maps (FA, directional-encoded color FA and apparent diffusion coefficient (ADC) maps) and the modeling for multiple fiber orientations were performed using the tensor and the CSD modules of the program MRtrix 0.2.10 (Tournier et al., 2007, 2012).

DEC st-TDI maps were generated as previously described (Calamante et al., 2012a), based on the probabilistic fiber-tracking results. First, the model for multiple fiber orientations was created using CSD with maximum harmonic order (l_{max}) of 6 (Tournier et al., 2004). We also included the CSD real ReY(m,l) and imaginary ImY(m,l)