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

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Highlights:

Mouse barrel cortex can be visualised in high definition using stTDI

stTDI produced superior barrel visualisation compared to conventional MRI and FA maps

stTDI is a novel imaging technique for 3D characterization of barrel cortex

stTDI can detect barrel changes resulting from infraorbital nerve cut

Keywords: mouse brain, barrel cortex, magnetic resonance, diffusion weighted imaging, track-density imaging, infraorbital nerve cut.

Abbreviations:

CSD	Constrained Spherical Deconvolution
DEC	Directionally Encoded Colour
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-track Track Density Imaging
TDI	Track Density Imaging
VPM	Ventral Posteromedial Thalamic nucleus
PMBSF	Posterior Medial Barrel Sub-Field

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-, T_2- 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.

1. 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, the large barrel field, referred to as the posterior medial barrel sub-field (PMBSF), barrels are arranged topographically in 5 rows (A-E) with 5 arcs (1-5). The cross-sectional area of PMBSF is approximately $1mm^2$, 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 380μm. 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 affected the accuracy measurements of individual barrels 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 have 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. (Calamante et al., 2012b) 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 grey matter, such as the molecular layer of the hippocampus, are more clearly visualized with stTDI (Calamante et al., 2012b). 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.

2. Materials and Methods:

2.1 Sample preparation

Twelve-week old adult C57BL/6J mice were anaesthetized and perfused with 4% paraformaldehyde containing 0.5mM Magnevist®. The brains were removed from the skull and placed in PBS containing 0.5mM 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 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 16.4T scanner (Bruker Biospin) using previously published protocols (Moldrich et al., 2010).

2.2 Ex-vivo MRI

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

The images described above were compared with the 30μm 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 30μm isotropic resolution with TE=20ms, NEX=12 (15.5h) and TE=12ms, NEX=8, acquisition time =13h. (2) T_1-weighted 3D spin-echo (SE) at 50μm isotropic resolution with TR/TE= 400/11 ms, NEX 1, acquisition time = 4h. (3) T_2-weighted 3D SE both at 50μm isotropic resolution with TR/TE= 1000/35 ms, NEX=2, acquisition time = 13h. (4) T_2-weighted 3D fast spin-echo (FSE) both at 50μm isotropic resolution with TR/TE= 1000/35 ms, RARE acceleration factor 4, NEX 4, acquisition time = 3h.

2.3 Ultra-high resolution cortical slab imaging

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

2.4 Diffusion parametric image reconstructions and fiber-tracking

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

DECst-TDI maps were generated as previously described (Calamante et al., 2012b), based on the probabilistic fiber-tracking results. First, the model for multiple fiber orientations was created using CSD with maximum harmonic order (*lmax*) of 6 (Tournier et al., 2004). We also included the CSD real ReY(m,l) and imaginary ImY(m,l) coefficient images for image analysis because at certain specific spherical harmonic order (l) and phase variation (m), they showed remarkable barrel contrasts.

Probabilistic fiber-tracking was performed using the following parameters: step-size=1/10 voxel-size, curvature= 45 degrees, and the track length between 2 and 10 times voxel size. The initial FOD threshold was 0.1 and fiber-tracking was terminated if the track exited the brain or if the FOD

amplitude was less than 0.01. To minimize the computational load for fiber-tracking, an additional volume mask with the dimensions of 3.96×10.56×5.28mm was created to cover the cortical area. This is possible with stTDI because tracks are only propagated for a short distance, and therefore tracks from outside this volume mask make a limited contribution to stTDI intensity.

To examine the effects of the number of tracks on visualization of cortical barrels, probabilistic fiber-tracking with 10 million tracks was performed 10 times for each masked brain dataset and stTDI maps were generated and averaged. This process is similar to generation of stTDI map using 100 million tracks, but less demanding to the computing hardware. To account for the effects of variations in the number of fibers in particular structures on the apparent noise level of stTDI maps, for each set of 10 stTDI maps, the variation map (standard deviation / mean) was calculated at each voxel. To test the effect of resolution, 65 million tracks were generated for the cortical slab data acquired at the two resolutions. stTDI and DEC stTDI maps were generated with a grid-size of 0.1 or 0.2 times of the in-plane resolution of the acquired image. The intensity unit of the stTDI map is expressed as the number of tracks per cubic grid-size.

To visualize the barrel cortex, the whole brain images were viewed in a transverse orientation (sections in a dorsal-ventral direction) and tilted along the rostral-caudal axis by 35°-55°, depending on the position of the brain in the scanner, and along the medial-lateral axis by 25°.

2.5 Comparison of barrel visualisation

To determine the clarity of barrel visualization from various image types and resolutions, the resulting MR images were rated by 8 researchers experienced in mouse brain imaging using a visual analog rating scale. They were all blinded to the image types, acquisition methods and TDI grid-resolutions. For each analysis, randomized 2D images in the optimal plane to visualize barrels were presented to the researchers to rate. Initially, a histology image showing barrel structure of the same brain (Section 2.6) was presented as a benchmark of maximum barrel image quality. The following tests were performed on these sets of images: (1) To test the effect of image types, GE, b0, ADC, ImY(*2,1*), ReY(*2,1*), FA-colour, FA which were compared to an average stTDI map of a single whole-brain sample. (2) To test the effect of full K-space vs. partial Fourier acquisitions on the quality of barrel definition, 10 of each acquisition type (20 in total) were compared using randomized barrel images. (3) To test the effect of the resolution of the raw HARDI data and the grid resolution to create stTDI maps, a series of 4 (raw/stTDI) images with 100/20μm, 100/10μm, 48/20μm, 48/10μm resolutions from a single 5mm cortical slab were compared. For these tasks, the researchers were asked to indicate a rating on a visual analog scale. Marking on the scale (0-6) indicated what the researcher thought of the image when compared to a histological image of the barrel field. A rating of 6 being the benchmark of image comparison. Examples of the images presented to the researchers are provided in Supplementary Figure 1.
For comparisons (1) and (3), the Kruskal-Wallis test was used with subsequent post-hoc multiple comparisons using Dunn's test. Comparison (2) was performed using the unpaired t-test with Welch's correction. All statistical analyses were performed using the program GraphPad Prism 5.0 (GraphPad Software inc., La Jolla, CA, USA).

2.6 Fluorescent labeling of barrel cortex

An adeno-associated virus expressing the tandem dimer (td) Tomato, a red fluorescent protein was injected into the ventral posteromedial thalamic nucleus (VPM) to label barrels in the somatosensory cortex, as previously described in rat (Wimmer et al., 2010). After labelling, mice were perfusion-fixed and MRI data were acquired as described above. The brains were then stored at 4°C in 1× PBS with 0.05% sodium azide until histological processing.

Brain samples (n=2) were placed inside a specifically designed holder to enable 40μm thick sections of the cortical slab to be obtained tangential to the barrels using a vibratome (VT 1000S, Leica Microsystems GmbH, Wetzlar, Germany). Sections were then mounted on charged glass slides (Superfrost Plus) and coverslipped with Fluorescence Mounting Medium (Dako Denmark A/S, Glostrup, Denmark). The mounted sections were examined on an epifluorescence microscope (Axio Imager 2, Carl Zeiss Microscopy GmbH, Jena, Germany) under the settings for 594 emission (Ex λ: 590nm; Em λ: 617nm), using an EC Plan-NeoFluar 5X objective (Carl Zeiss). High-resolution images with 1.29x1.29μm in-plane resolution were obtained using a monochromatic CCD camera (AxioCam HRm, Zeiss) attached to the microscope.

2.7 Registration of histological slices to TDI maps

A MRI-guided histology reconstruction method developed in our laboratory (Yang et al., 2012) was adapted to stack the digitized sections into a histological volume and to register it to the MRI data. The histological data comprised 10 epifluorescence sections, resampled to the same voxel size as the stTDI volume. To facilitate intensity-based registration, stTDI intensity values were inverted before registration. Landmark-based registration was first performed in MIPAV (http://mipav.cit.nih.gov/) to account for large differences in the orientation and field of view of the two datasets. Rigid and affine registrations were performed in sequence in MatLab® (MathWorks, Natick, MA). The corresponding stTDI slices registered to the histological sections are shown in Supplementary Figure S2.

We measured the Euclidean distances between two barrel centres shown by the stTDI method in comparison to barrels visualized using virus mediated fluorescent protein expression. Barrel centres were determined in stTDI map by first displaying the plane containing the barrel in an oblique orientation such that this plane displayed the largest area of the PBMSF. Subsequently, the longest axis of the barrel was searched and the centre of the barrel was then determined by finding the point that produced the longest orthogonal axis to the septum. The Euclidean distance between two barrels was measured from these centre points. The Euclidean distances between C1-D1, C1-E1, C1-C2, and D1-D2 were calculated for the right barrel cortex from three 20μm resolution stTDI maps of the whole brain datasets and from corresponding fluorescent labelled histological specimens of the same brain sample.

2.8. Assessment of changes in barrel conformation following infraorbital nerve cut

To assess the potential of stTDI to detect changes in the barrel cortex formation, unilateral infra-orbital (whisker) nerve cuts (n=3) were performed with C57BL/6J mice at age postnatal day 2 (P2). In this procedure, the barrels on the left hemisphere (contralateral to the nerve cut site) would be affected and the ipsilateral barrels could be used as the control (Waite and Cragg, 1982). Mouse pups were anaesthetized by hypothermia and the right infraorbital nerve was exposed and transected by making an incision just behind the whisker pad. The wound was closed with Vetbond (3M). After the operation, the pups were revived and returned to their dams. At P30, ex-vivo fixed brains were prepared using PFA fixation (Section 2.1) and incubated with 0.5 mM Magnevist® over 4 days. The samples were imaged using 15h 100μm HARDI and 2.5h 30 μm 3D GE (Section 2.2) and processed using stTDI (Section 2.4).

Post MRI, the brains were dissected and flattened between two slides placed approximately 1mm apart and fixed in 4% PFA in PBS at 4°C for 1-2 weeks. Following fixation, the flattened cortices were sectioned using a vibrating microtome (Leica Biosystems VT1100s, Nussloch, Germany) at 50μm thickness. Sections were then mounted onto gelatin-coated slides, air-dried and re-fixed for 15 minutes in 4% PFA in PBS. After brief rinsing with PBS, sections were blocked for 2 hours in PBS blocking solution containing 10% normal goat serum (Vector Laboratories, CA) and 0.2% Triton X-100 (Sigma-Aldrich Australia). Subsequently, they were incubated overnight at room

temperature in anti-vesicular glutamate transporter 2 (VGLUT2) primary antibody (1:750; Synaptic Systems GmBH, Göttingen, Germany) diluted in the blocking solution. After washing in PBS for 4 × 20 minutes, sections were incubated for 3 hours in goat-anti-rabbit IgG (Alexafluor 555, 1:500; Invitrogen Life Technologies, Mulgrave, Australia). After the desired staining was achieved, sections were counterstained with DAPI (Invitrogen) and washed with PBS for 3 × 10 minutes before coverslipped with ProLong® Gold Antifade Reagent (Invitrogen). Fluorescence images were acquired using a 20 x 0.8 NA Plan-apochromat objective on a V-slide slide-scanning microscope with Metafer 4 software (Metasystems, Heidelberg, Germany). Slide images were viewed and analyzed using Imaris 7.5 (Bitplane, CT, USA).
PMBSF areas shown by stTDI and VGLUT2 immunofluorescence were compared between the two hemispheres to assess barrel changes resulting from the nerve cut.

3. Results:

3.1 Lack of visualization of the barrel structures using 3D GE, SE and FSE images

3D GE images of the samples prepared using Magnevist® concentrations at 0.5 mM (Figure 1A, B) and 2.5 mM (Figure 1C, D) and incubation times of 1 week (Figure 1A, C) and 1 month (Figure 1B, D) could not produce consistent enhanced visualization of the cortical barrel structures. These structures were also invisible in individual brains used to generate the Australian Mouse Brain Mapping Consortium (AMBMC) adult C57BL/6J mouse brain model (Ullmann et al., 2012). Although signal to noise and contrast improvement from averaging 18 brains to create the 15μm minimum deformation atlas have enhanced visualization of structures such as the thalamic nuclei and cortical layers, which were initially indistinguishable in the individual brains, barrel structures could not be visualised on the minimum deformation atlas (Figure 1 E).

3D GE, SE and FSE images at 50μm resolution with T_1/T_2*-, T_1 and T_2-weightings, repetitive excitations and long acquisition times (up to 15.5h) created datasets with higher SNR compared to the 100μm HARDI data, but still did not reveal barrel structures. The 2D slices corresponding to layer IV of the barrel cortex area and the acquisition parameters from these long acquisitions are shown in Supplementary Figure S3.

3.2 Visualization of the barrel structure using stTDI

The ability to visualize barrel structures was examined after each processing step in stTDI map reconstruction. Examination of the spherical harmonic coefficient images associated with each spherical harmonic order (l = 0, 2, 4, 6) and phase variation factor ($0 \leq m \leq l$) showed that the barrel structures were not visible at l=0 (Figure 2C) but become recognized at l=2 and m=1, albeit with low spatial resolution (Figure 2E, G). By comparison, the contrast between barrels and surrounding cortex in FA images was less uniform (Figure 2B). Barrel structures were not clearly observed in ADC and eigenvalue maps (data not shown). Compared to the CSD coefficients, barrels could be distinguished much more clearly on DEC stTDI and stTDI maps (Figure 3A, B). The stTDI maps also produce better barrel visualization than the FA and colour FA maps (Figure 3C, D). In stTDI maps, individual barrels were hypointense (stTDI map intensity 54.08±7.02; mean±standard deviation) compared to the surrounding septa (93.17±9.73), reflecting a lower number of short-length streamlines in the barrels compared to the surrounding tissue.

Comparison between image types (Figure 4A) indicated that the barrels were least clearly visualized on the GE, b0 and ADC, whereas CSD ImY(2,1), ReY(2,1), FA, and FA-colour maps were rated with intermediate clarity. ImY(2,1) and stTDI map were both rated significantly better than GE, b0 and ADC barrel visualization. The ratings, however, varied widely between observers

for ImY(2,1), ReY(2,1), FA and FA-color maps, indicating significant ambiguity in determining the barrel structures. Non-parametric statistical test confirmed the vast improvement in barrel visualization using stTDI compared to ReY(2,1), FA, and FA-colour maps. Additionally, the stTDI maps from full K-space acquisition data appeared to have small but significant improvement for the quality of the barrel definition over those reconstructed from partial Fourier acceleration (Figure 4B). These barrel images can be seen in Supplementary Figure S1 Task 3.

3.3 Impact of acquired diffusion MRI resolution on stTDI delineation of the barrel structure

Comparison of TDI maps from the cortical slab generated from 100 and 48μm isotropic resolution data showed the critical influence of acquisition resolution on the quality of stTDI maps. stTDI maps reconstructed at 10μm resolution using HARDI data acquired at 48μm (Figure 5D) produced superior barrel maps compared to those generated from 100μm data (Figure 5A, B). The results of the visual ratings suggest that there was no significant improvement in barrel visualization from processing the 100μm HARDI data at 10μm versus 20μm grid resolution (Figure 4C), and no significant improvement for barrel visualization was detected from the 48μm HARDI data at 10μm versus 20μm grid resolution despite improved consistency between raters. The visualization of the barrel structures on ultra-high resolution stTDI (Figure 6B, C) was also superior to high-resolution conventional FA maps (Figure 6A, E, F) and ultra-high resolution GE images (Figure 6D).

It should be also noted that the super-resolution step was important to achieve good visualization, particularly for the 100μm resolution HARDI data, given that this acquired resolution is low compared to the barrels. The improvements with super-resolution are illustrated in Supplementary Figure S4, where sTDI maps *without* super-resolution (i.e. at the native acquired resolution) and *with* super-resolution are shown. The barrels can be visualized more clearly with super-resolution; in particular, the barrels can be barely resolved using sTDI without super-resolution for the 100μm HARDI.

3.4 Effect of number of tracks in visualization of barrel structures

Improved contrast between the barrels and the surrounding cortex was observed in the averaged 20μm stTDI maps (Figure 7A, D, E, H, I) compared to the individual stTDI maps (Figure 7 panels 1-9). In the individual maps, the average standard deviation of the intensity of the barrel was approximately 7.4±1 (Figure 7B). This was reduced by 46% to 3.9±0.8 in the averaged 10 stTDI maps, and a similar level of reduction to the average standard deviation was also observed for the septa. On the other hand, the mean intensities of the barrel and the septa were unchanged upon averaging.

The variation map calculated from ten individual stTDI runs (Figure 7C, G, K) shows that the individual barrels have small stTDI variations (0.13±0.03), which is comparable to other grey matter structures such as the CA1-3 hippocampus (0.13±0.04) and dentate gyrus (0.15±0.03). These results indicate that with increasing number of tracks, the barrel structures have lower regional TDI map intensity variability, and thus can be confidently determined at a similar level to the other brain structures.

3.5 Barrel identification: Validation of stTDI with histological images

To facilitate sectioning of the brain in the tangential plane for 3D reconstruction, the barrel cortex was cut in a specifically designed holder (Figure 8A). Our objective was to help reduce distortion/stretching in the histological slices, which result from flattening of the cortical slab prior to sectioning. Initial trials to reconstruct 3D histology volume using slice-by-slice rigid body registration produced poor results due to the absence of landmarks in somatosensory cortex. This registration method produced a curved barrel column with in-plane rotations of the barrel field

(result not shown). The 3D volume reconstruction was remarkably improved when the registration used the landmarks set from the 20µm stTDI maps of the masked whole brain HARDI data (Supplementary Figure S2). The result of 3D volumetric PMBSF delineations from stTDI map and the corresponding reconstructed histology volume is shown in Supplementary Video S5.

With reference to fluorescent histology images from the same animal, stTDI maps showed superior visualisation of the barrel compared to FA or FA colour maps (Figure 8B, C, D). The hyperintense staining of individual barrels in the fluorescence images fits well to individual barrels identified on stTDI images. However, the barrel pattern observed in the FA and colour FA maps had a significantly lower clarity and showed irregular patterns which did not match histological images. In FA maps, the size of the barrels was reduced and the septal regions were correspondingly enlarged rendering correlation with histology difficult.

The mean (± standard deviation) Euclidean distances for the right barrel cortex (n=3) between C1-D1, C1-E1, C1-C2, and D1-D2 for the 20µm stTDI map were 346 ±29µm, 590 ±34µm, 244 ±9µm and 243 ±18µm, respectively. Similar results were obtained from the fluorescence images of the same animals; they were 362 ±16µm, 589 ±8µm, 224 ±19µm and 221 ±1µm, respectively. Similar results were obtained from the best quality 10µm stTDI map of a different sample, with the distances of C1-D1=340µm, C1-E1=640µm, C1-C2=270µm and D1-D2=210µm.

Measurements in the PMBSF area using high-resolution fluorescent images showed that the widths of the septa range from 10-20µm. Such measurements were difficult to reliably obtain from stTDI maps as they are in the limit (1-2 super-resolution voxels) of the image resolution.

3.6 Changes in barrel conformation following infraorbital nerve cut

Distinct changes in the barrel field post infra-orbital nerve cut could be detected using the stTDI method (Figure 9). In this experiment, barrel deletions were observed in PMBSF areas in rows A-C (sample 1, Figure 9E), and rows A-B (sample 2, Figure 9G). These observations matched with immunofluorescence staining for slices of the same samples using VGLUT2 antibody (Figure 9F, H). In comparison, the barrel field appeared unaffected ipsilateral to the nerve cut (control areas). Variability in the change to the barrel field observed in our samples may be due to incomplete transection or to partial repair following infraorbital nerve injury as regeneration of a large portion of the myelinated fibers (24-56%) has previously been observed in this experimental preparation (Waite and Cragg, 1982). stTDI can detect distinct deletions of the barrels and highlights the application of stTDI to visualize brain plasticity in the barrel field.

4. Discussion

In this study, we found that visualization of barrels in the *ex-vivo* mouse brain is greatly enhanced with super-resolution stTDI compared to conventional FA maps, GE, b0 and ADC maps. Visualization was superior on 10µm stTDI maps from 48µm HARDI data compared to 10µm stTDI maps reconstructed from 100µm HARDI data. The barrels appeared smaller and the septa wider in the lower resolution HARDI data, indicating that the stTDI method is affected by the quality and the resolution of raw HARDI data. This is expected given that the smaller the acquired voxel size, the lower the partial volume effect, and therefore the easier it will be for fiber-tracking to resolve the barrels using diffusion information contained in the surrounding voxels. Finally, the barrel structure on stTDI corresponded well with reconstructed histology data, demonstrating the accuracy and sensitivity of the technique in identifying this structure.

Demarcation of cortical 'barrels' using diffusion imaging at such high spatial resolution is unprecedented, and provides an additional valuable mapping tool for comparisons across different mouse models of health and disease. Segmentation of barrels and septa can be used to define cortical and thalamic connectivity patterns (Petreanu et al., 2009; Wimmer et al., 2010; Woolsey and Van der Loos, 1970). stTDI defined layer IV barrels may also be used to locate cortical columns, archetype circuits that represent the fundamental cortical elements (Kuljis, 1992; Mountcastle, 1957; Woolsey and Van der Loos, 1970).

4.1. Ultra-high resolution diffusion MRI for mouse brain TDI.

In human TDI studies, HARDI datasets are typically acquired with 2D diffusion-weighted echo planar imaging (EPI) at ~2-2.5mm isotropic resolution, using 60 diffusion encoding directions in ~10 minutes. Such datasets have been used to visualize white matter structures (Calamante et al., 2011; Calamante et al., 2010; Calamante et al., 2012c; Cho et al., 2013) and have also been applied to the identification of sub-cortical grey matter structures, such as in the thalamus (Calamante et al., 2012a).

The use of TDI for mouse brains, however, has been limited to a single *ex-vivo* study (Calamante et al., 2012b). High quality high spatial resolution HARDI is critical to resolve many of the very fine structures of the mouse brain. Compared to the 30-micron resolution anatomical T_1/T_2* images, the 100-micron HARDI data have not only significantly lower spatial resolution (by 38 times) but also a longer acquisition time (by 3 times). HARDI data could be zero-filled prior to Fourier transformation to increase the accuracy for FOD spherical harmonics (SH) interpolation during fiber-tracking (Tournier et al., 2012), but this does not increase the resolution of the acquired data.

In the present study, the long acquisition times for HARDI data was also due to the relatively large number of diffusion gradient directions (30 directions) required for sufficient angular resolution with processing CSD with *lmax*=6 (a minimum of 28 directions is required). These acquisition parameters were chosen to balance spatial resolution and signal-to-noise at high b-values to maintain high FOD angular resolution given a moderate number of acquired diffusion encoding directions. In contrast, when resolving multiple fiber populations within a voxel is not of interest, ultra-high spatial resolution diffusion tensor imaging of a whole mouse brain can be acquired at 43μm isotropic resolution with small number of gradient directions (≤16) and low b-values (≤1500 s/mm^2) in approximately 30h at 11.7 or 9.4T (Aggarwal et al., 2012; Jiang and Johnson, 2010, 2011).

At 16.4T, we found that high-resolution 3D DWI EPI suffered from image distortion. This presented difficulty in implementing 3D-DWI EPI to reduce the acquisition time (Aggarwal et al., 2012; D'Arceuil and de Crespigny, 2007). A significant number of phase encoding segmentations were required to reduce the TE to bring the image quality to the same level of 3D DWI spin echo. The distortion problem would be exacerbated for *in vivo* imaging, where larger effects were observed in tissue boundaries and near air cavities with lengthy imaging time due to respiration gating to reduce motion artefacts.

4.2 The mouse barrel field is difficult to visualise using T_1, T_2 and T_2-weighted imaging*

The methodology for sample preparation and 3D high-resolution GE parameter imaging sequence used in this experiment is similar to that used to develop the AMBMC atlas (Ullmann et al., 2012). However, in both cases and also at various contrast agent concentrations/incubation periods, the barrel structure could not be reliably visualised. The 15μm AMBMC model averaged from 18 samples did not provide improved barrel visibility, possibly due to the minimal contrast in the original individual data and normal inter-brain variability. Differences in perfusion fixation process such as buffer pressure, flow rate and the amount of fixative and contrast agent reaching the brain

has been described to significantly affect the tissue contrast observed by MRI (Cahill et al., 2012). In addition, dilation of the cortical vessels during the fixation perfusion process has been reported to produce black spots in T_1/T_2*-weighted GE images (Cleary et al., 2011), obscuring the visualization of the barrel structures. Indeed, we observed similar problems in many of the samples acquired using this sequence. We have attempted to minimize these issues by delivering the same amount of anesthetics, volume and flow rate of the fixative. Nevertheless, we found that the resulting barrel contrasts were highly variable in our samples imaged using T_1/T_2*-weighted GE sequence. The problems associated with capillary artefacts, however, were not visible in the final AMBMC minimum deformation atlas model, where variability was most likely removed by averaging during the model calculations (Ullmann et al., 2012).

High-resolution images with variable T_1, T_2 and T_2* weightings and longer acquisition times produced images with higher SNR but did not improve the contrast required to reveal individual barrel structures. These results were in contrast to HARDI acquisition, where 30 diffusion directions were sampled, which provided complex directional diffusion information for processing using CSD/TDI. This observation suggests that the barrel structures have distinct properties that can only be efficiently distinguished using diffusion-weighted imaging and TDI methods.

4.3 Visualization of the barrel structure using stTDI map

In contrast to T_1, T_2 and T_2* imaging, we found that stTDI mapping was less impacted by perfusions and contrast agent concentrations, instead all brains produced excellent barrel visualizations. Compared to other conventional MRI methods tested in this paper, it appears that the combination of HARDI, CSD and the super-resolution stTDI methods are vital to produce the contrast needed to differentiate the dense afferent thalamo-cortical axonal projections/terminals in the barrel in comparison to the septum and surrounding tissue (Wimmer et al., 2010). The barrel cortex is rich in crossing fibers that are made up of axons and dendritic arborizations arranged within the cortical layers; however local connectivity does not contain highly ordered myelinated axons. With a relatively large MRI voxel size compared to that of individual barrels, the partial volume effect is significant. On its own, HARDI when processed using DTI cannot reveal individual barrel structures due to significant partial volume effect between the barrels and the septa and the inability to resolve intra-voxel directionality; this area thus appears only with relatively low diffusion directionality (approximately isotropic). CSD was able to resolve this intra-voxel directionality, although the resulting FOD amplitudes were generally low. The CSD coefficient map itself, although it can reveal the barrel pattern to a certain degree, did not produce high quality barrel definition due to its limited spatial resolution. To exploit low FOD amplitude information, a large number of probabilistic streamlines need to be generated. The barrel centers have smaller FOD compared to the surrounding septal area and thus contain a smaller number of tracks. High-resolution stTDI grids were critical for mapping directionality information from surrounding voxels to reveal barrel structure.

stTDI maps were required to clearly delineate individual barrels, however there are variations in the apparent thickness of the septum depending on the raw acquisition and stTDI resolutions. The resolution of the 100μm whole-brain HARDI datasets is much too large for the septa (10-20μm), but marginally sufficient to cover the cross sections of the large barrels in PMBSF (200-300μm). This would result in significant partial-volume effects at the periphery compared to the centre of the barrels. In the DTI parametric maps, the quality of the FA maps was severely affected, where the barrel appearance was variable in intensity and shape and barrels were often difficult to identify. The super-resolution gridding applied in TDI can alleviate this problem to some degree. The centre of the barrels have the lowest number of streamlines and FOD compared to the surrounding tissue, resulting in hypointense regions in the stTDI maps. Compared to the 10μm stTDI maps, the borders

of the septum appeared less sharp in the 20μm stTDI maps and affecting the accuracy of barrel measurements.

4.4 Validation of stTDI barrel field with histology

This study used the methods of injection of adeno-associated virus expressing fluorescent protein specifically into the VPM (Wimmer et al., 2010) and fluorescent immunohistochemistry using vGLUT2 antibody (Liguz-Lecznar and Skangiel-Kramska, 2007) to label the barrel field. We observed that these methods have revealed the barrel structures much more clearly compared to alternative staining methods.

A recent study of barrel cytoarchitecture using 3D reconstruction of histology indicated that it was difficult to measure the barrel centre distances and volume accurately from such slices (Egger et al., 2012), due to systematic errors from the curvatures of the cortex and low precision in histological slice preparation. Visualization of individual barrels using stTDI map avoids this problem, as intact brain or cortical slab can be imaged as a whole. Additionally, the barrel stTDI map can be used as a reference to reconstruct 3D histological volume to enable a more accurate measurement of the barrel structures.

4.5 Consideration of using stTDI over histology for barrel visualization

Given the intrinsic higher image resolution and clarity of histological images, better quality measurements such as barrel volumes, column angles and septum thickness can theoretically be obtained from well-reconstructed 3D histology compared to TDI. However, MRI does offer various important advantages over histology. The whole tissue structure can be preserved, so the data is acquired in a fully 3-dimensional way, and visualized in various orientations without tissue distortion from sectioning and flattening. Furthermore, the images are intrinsically co-registered to other image contrast available by MRI, as well as the ability to analyze from the same diffusion MRI data important aspects of white matter connectivity (for example long-range fiber-tracking between somatosensory pathways). Given the relatively low cost of overnight imaging at many MRI centers, the relatively long HARDI scanning is not a major limitation. It should be also noted that 3D reconstruction of the histological images without the aid of TDI resulted in incorrect geometry, where the barrel columns appeared flat (instead of following the curvature of the cortex) in the coronal plane (along the rostro-caudal axis), and were irregularly rotated transversely when viewed in the antero-posterior plane. With the aid of TDI, barrel histology image reconstruction becomes remarkably better defined and accurate, and this could be one key application of TDI to complement histology. To date, block face imaging to visualize the barrel cortex has not been validated and may present obstacles as barrel visualization requires antibody staining which is difficult to obtain using a whole brain preparation. The TDI technique therefore provides a very powerful (and arguably unique) MRI method to characterize the barrel structures, and complement the information available by histology.

Conclusion: This study is the first to show a robust visualization of the barrel fields in the mouse brain using MRI. 3D structure of the barrel fields can be clearly and reliably visualized using the super-resolution stTDI technique. These maps produced superior contrast compared to DTI parametric images and conventional MRI and provide a significant advance in our ability to map structural and functional changes in the somatosensory barrel cortex caused by genetic or acquired lesions.

Acknowledgements We are grateful to the National Health and Medical Research Council (NHMRC) of Australia, and the Australian Research Council (ARC) for their support. The Florey

Institute of Neuroscience and Mental Health is supported by Victorian State Government infrastructure funds. We thank the Queensland state government support for the operation of the 16.4T through the Queensland NMR Network. JUY was supported by the Chilean National Scholarship. We thank Prof. LJ Richards for critical reading of the manuscript and histology.

5. Reference

Aggarwal, M., Jones, M.V., Calabresi, P.A., Mori, S., Zhang, J., 2012. Probing mouse brain microstructure using oscillating gradient diffusion MRI. Magn Reson Med 67, 98-109.

Cahill, L.S., Laliberte, C.L., Ellegood, J., Spring, S., Gleave, J.A., Eede, M.C., Lerch, J.P., Henkelman, R.M., 2012. Preparation of fixed mouse brains for MRI. Neuroimage 60, 933-939.

Calamante, F., Oh, S.H., Tournier, J.D., Park, S.Y., Son, Y.D., Chung, J.Y., Chi, J.G., Jackson, G.D., Park, C.W., Kim, Y.B., Connelly, A., Cho, Z.H., 2012a. Super-resolution track-density imaging of thalamic substructures: Comparison with high-resolution anatomical magnetic resonance imaging at 7.0T. Hum Brain Mapp.

Calamante, F., Tournier, J.D., Heidemann, R.M., Anwander, A., Jackson, G.D., Connelly, A., 2011. Track density imaging (TDI): validation of super resolution property. Neuroimage 56, 1259-1266.

Calamante, F., Tournier, J.D., Jackson, G.D., Connelly, A., 2010. Track-density imaging (TDI): super-resolution white matter imaging using whole-brain track-density mapping. Neuroimage 53, 1233-1243.

Calamante, F., Tournier, J.D., Kurniawan, N.D., Yang, Z., Gyengesi, E., Galloway, G.J., Reutens, D.C., Connelly, A., 2012b. Super-resolution track-density imaging studies of mouse brain: comparison to histology. Neuroimage 59, 286-296.

Calamante, F., Tournier, J.D., Smith, R.E., Connelly, A., 2012c. A generalised framework for super-resolution track-weighted imaging. Neuroimage 59, 2494-2503.

Cho, Z.H., Calamante, F., Chi, J.G., 2013. 7.0 Tesla MRI Brain White Matter Atlas. Panmun Book Company; Seoul, South Korea.

Cleary, J.O., Wiseman, F.K., Norris, F.C., Price, A.N., Choy, M., Tybulewicz, V.L., Ordidge, R.J., Brandner, S., Fisher, E.M., Lythgoe, M.F., 2011. Structural correlates of active-staining following magnetic resonance microscopy in the mouse brain. Neuroimage 56, 974-983.

D'Arceuil, H., de Crespigny, A., 2007. The effects of brain tissue decomposition on diffusion tensor imaging and tractography. Neuroimage 36, 64-68.

Daw, M.I., Ashby, M.C., Isaac, J.T., 2007. Coordinated developmental recruitment of latent fast spiking interneurons in layer IV barrel cortex. Nat Neurosci 10, 453-461.

Dorr, A.E., Lerch, J.P., Spring, S., Kabani, N., Henkelman, R.M., 2008. High resolution three-dimensional brain atlas using an average magnetic resonance image of 40 adult C57Bl/6J mice. Neuroimage 42, 60-69.

Egger, R., Narayanan, R.T., Helmstaedter, M., de Kock, C.P., Oberlaender, M., 2012. 3D Reconstruction and Standardization of the Rat Vibrissal Cortex for Precise Registration of Single Neuron Morphology. PLoS Comput Biol 8, e1002837.

Gleave, J.A., Wong, M.D., Dazai, J., Altaf, M., Henkelman, R.M., Lerch, J.P., Nieman, B.J., 2012. Neuroanatomical phenotyping of the mouse brain with three-dimensional autofluorescence imaging. Physiol Genomics 44, 778-785.

Gonzalo-Ruiz, A., Sanz-Anquela, M.J., Lieberman, A.R., 1995. Cholinergic projections to the anterior thalamic nuclei in the rat: a combined retrograde tracing and choline acetyl transferase immunohistochemical study. Anat Embryol (Berl) 192, 335-349.

Grinvald, A., Hildesheim, R., 2004. VSDI: a new era in functional imaging of cortical dynamics. Nat Rev Neurosci 5, 874-885.

Jiang, Y., Johnson, G.A., 2010. Microscopic diffusion tensor imaging of the mouse brain. Neuroimage 50, 465-471.

Jiang, Y., Johnson, G.A., 2011. Microscopic diffusion tensor atlas of the mouse brain. Neuroimage 56, 1235-1243.

Johnson, G.A., Badea, A., Brandenburg, J., Cofer, G., Fubara, B., Liu, S., Nissanov, J., 2010. Waxholm space: an image-based reference for coordinating mouse brain research. Neuroimage 53, 365-372.

Kuljis, R.O., 1992. Vibrissaeless mutant rats with a modular representation of innervated sinus hair follicles in the cerebral cortex. Exp Neurol 115, 146-150.

Land, P.W., Simons, D.J., 1985. Cytochrome oxidase staining in the rat SmI barrel cortex. J Comp Neurol 238, 225-235.

Liguz-Lecznar, M., Skangiel-Kramska, J., 2007. Vesicular glutamate transporters VGLUT1 and VGLUT2 in the developing mouse barrel cortex. Int J Dev Neurosci 25, 107-114.

Ma, Y., Hof, P.R., Grant, S.C., Blackband, S.J., Bennett, R., Slatest, L., McGuigan, M.D., Benveniste, H., 2005. A three-dimensional digital atlas database of the adult C57BL/6J mouse brain by magnetic resonance microscopy. Neuroscience 135, 1203-1215.

Moldrich, R.X., Pannek, K., Hoch, R., Rubenstein, J.L., Kurniawan, N.D., Richards, L.J., 2010. Comparative mouse brain tractography of diffusion magnetic resonance imaging. Neuroimage 51, 1027-1036.

Mountcastle, V.B., 1957. Modality and topographic properties of single neurons of cat's somatic sensory cortex. J Neurophysiol 20, 408-434.

Petersen, C.C., 2007. The functional organization of the barrel cortex. Neuron 56, 339-355.

Petreanu, L., Mao, T., Sternson, S.M., Svoboda, K., 2009. The subcellular organization of neocortical excitatory connections. Nature 457, 1142-1145.

Simons, D.J., Woolsey, T.A., 1979. Functional organization in mouse barrel cortex. Brain Res 165, 327-332.

Tournier, J.D., Calamante, F., Connelly, A., 2007. Robust determination of the fibre orientation distribution in diffusion MRI: non-negativity constrained super-resolved spherical deconvolution. Neuroimage 35, 1459-1472.

Tournier, J.D., Calamante, F., Connelly, A., 2012. MRtrix: Diffusion tractography in crossing fiber regions. Int. J. Imaging Syst. Technol. 22, 53-66.

Tournier, J.D., Calamante, F., Gadian, D.G., Connelly, A., 2004. Direct estimation of the fiber orientation density function from diffusion-weighted MRI data using spherical deconvolution. Neuroimage 23, 1176-1185.

Ullmann, J.F., Keller, M.D., Watson, C., Janke, A.L., Kurniawan, N.D., Yang, Z., Richards, K., Paxinos, G., Egan, G.F., Petrou, S., Bartlett, P., Galloway, G.J., Reutens, D.C., 2012. Segmentation of the C57BL/6J mouse cerebellum in magnetic resonance images. Neuroimage 62, 1408-1414.

Voutsinos-Porche, B., Knott, G., Tanaka, K., Quairiaux, C., Welker, E., Bonvento, G., 2003. Glial glutamate transporters and maturation of the mouse somatosensory cortex. Cereb Cortex 13, 1110-1121.

Waite, P.M., Cragg, B.G., 1982. The peripheral and central changes resulting from cutting or crushing the afferent nerve supply to the whiskers. Proc R Soc Lond B Biol Sci 214, 191-211.

Welker, C., 1976. Receptive fields of barrels in the somatosensory neocortex of the rat. J Comp Neurol 166, 173-189.

Wimmer, V.C., Bruno, R.M., de Kock, C.P., Kuner, T., Sakmann, B., 2010. Dimensions of a projection column and architecture of VPM and POm axons in rat vibrissal cortex. Cereb Cortex 20, 2265-2276.

Woolsey, T.A., Van der Loos, H., 1970. The structural organization of layer IV in the somatosensory region (SI) of mouse cerebral cortex. The description of a cortical field composed of discrete cytoarchitectonic units. Brain Res 17, 205-242.

Yang, Z., Richards, K., Kurniawan, N.D., Petrou, S., Reutens, D.C., 2012. MRI-guided volume reconstruction of mouse brain from histological sections. J Neurosci Methods 211, 210-217.

Yu, X., Glen, D., Wang, S., Dodd, S., Hirano, Y., Saad, Z., Reynolds, R., Silva, A.C., Koretsky, A.P., 2012. Direct imaging of macrovascular and microvascular contributions to BOLD fMRI in layers IV-V of the rat whisker-barrel cortex. Neuroimage 59, 1451-1460.

Figure Legends

Figure 1. Variability of barrel contrast and visibility in 3D gradient echo images. (A-D) Systematic variations of Magnevist® concentrations used for staining 0.5 mM (A, B) and 2.5 mM (C, D) and the length of incubation time: 1 week (A, C) and 1 month (B, D) could not produce consistent enhanced visualization of the barrel structures. (E) AMBMC C57BL/6J atlas of the corresponding barrel cortex area. Representative images of the samples tested were shown in a tangential slice (~35 degrees) to the horizontal axis. The capillaries of the cortex appeared as intense black pixels in the images. Scale bars are 0.2 mm.

Figure 2. Diffusion parametric images revealed distinct barrel structure arrangement. As the 3D GE image, the b0 of the DWI-spin echo image, which is T_1 and T_2 weighted, failed to provide the barrel contrast. The overall barrel field can be seen in the FA map, where high number of terminating thalamocortical afferent axons in the barrels produces higher degree of diffusion anisotropy compared to the surrounding grey matter regions. The barrel pattern can also be detected with the CSD coefficient corresponding to the real and imaginary $Y(l = 2, m = 1)$ term of the spherical harmonic (SH) expansion. The contrasts and delineation of the barrels shown in the FA maps, however, have much lower quality compared to the two CSD coefficient images. The following images are shown: b0 image (A), FA map (B), CSD coefficient maps: [0]Y(0,0) (C), [1]Im(2.2) (D), [2]ImY(2,1) (E), [3]Y(2,0) (F), [4]ReY(2,1) (G), [5]ReY(2,2) (H). Scale bars are 0.2 mm.

Figure 3. Visualisation of the cortical barrel field using stTDI maps. (A) DEC-stTDI, (B) stTDI, (C) FA-colour and (D) FA maps. Tangential slices are taken from the same position, displayed using MRView. The RGB colour indicates direction (Red: dorsal-ventral, green: medial-lateral, blue: rostral-caudal). This TDI dataset was reconstructed from 15h HARDI acquisition at 100μm resolution. Scale bar is 0.2 mm.

Figure 4. User's rating for the quality of barrel visualisation. User's perception on the clarity of barrel structure is dependent on image types (A) and the resolutions of HARDI data and stTDI maps (C). Comparison between full K-space and partial Fourier acceleration acquisitions to the quality of definition for the barrel (B). The significance level $p<0.05$, 0.01 and 0.001, are indicated by *, **, ***, respectively.

Figure 5. Ultra-high resolution HARDI data influenced the quality of reconstructed TDI-maps. A 3mm cortical slab was imaged using ultra-high resolution 3D HARDI acquisitions at 100 micron (A-B) and 48μm (C-D). Fiber-tracking was performed after CSD (l_{max}=6) with 65 million

tracks. stTDI maps were reconstructed using 20 μm (A,C) and 10 μm grids (B,D). Scale bars are 0.2 mm.

Figure 6. Comparison of stTDI, FA maps and gradient echo images acquired using ultra-high resolution microimaging. A cortical slab containing barrel cortex was prepared to fit into a 5mm solenoid coil. (A, B, D, E) are matching tangential slice images of the sample. (A) 48μm resolution colour-encoded FA map, (B) 48mm HARDI with 10μm grid resolution DEC-stTDI map, (D) 10 μm resolution 3D GE image, and (E) 48μm resolution FA map. Individual barrels can be seen as hypointensity areas in the DEC-stTDI and FA map. (C) and (F) are DEC-stTDI and FA map coronal slice images taken at the plane marked by dashed line in (B), showing the arrangement of the barrel nuclei in layer IV of the cortex. Scale bar is 0.2 mm.

Figure 7. Combination of multiple trials of fiber-tracking and stTDI reconstructions can be used to improve the delineation of the barrels. 10 stTDI maps were calculated where each dataset was generated with 10 million tracks calculated using probabilistic streamlines from the same acquired HARDI dataset (9 of 10 maps (1-9) are shown in the figure). The delineation of the barrel improves as the TDI maps from 10 runs are averaged (A). A comparison between 10 stTDI runs showed that the cortex area has low standard deviation (STDEV) (B), with the variation (STDEV/mean) in the barrels is higher than the surrounding grey matter tissue (C). (D-G) and (H-K) showed the average of DEC stTDI and stTDI maps, and STDEV and variation maps in the coronal and transverse orientations. A relatively high variation compared to the surrounding tissue is also observed in the hippocampus. Scale bars are 0.2 mm and the unit is the number of tracks per $(20\mu m)^3$ grid.

Figure 8. Correlation of the barrel cortex structure obtained from whole-brain diffusion MRI and histology. (A) To cut the cortical slab, the brain was placed in a holder to tilt its position along the rostral-caudal axis by 35 degrees and along the medial lateral axis by 25 degrees. (B) Coronal slice of a mouse brain showing the position of the barrel cortex and the injection site of adeno-associated virus expressing td-Tomato fluorescent protein at the VPM. The dashed lines are 35 degrees angled from the horizontal plane. (C) Td-tomato fluorescent histology tangential slice. (D) Registered whole-brain stTDI map to the fluorescence image (C) with the arc/row designations shown. Note that the contrast of the stTDI image was inverted to aid registration and visualisation. This TDI dataset was reconstructed from 15h HARDI acquisition at 100μm resolution. Scale bars are 0.2 mm.

Figure 9. Detection of changes in barrel field post infraorbital transection. Mice (M1, M2, M3) were subjected to a unilateral partial infraorbital surgery at P2 and *ex-vivo* brains (P30) were imaged with *ex-vivo* stTDI (A, C, E, G) followed by immunofluorescence staining using VGLUT2 (B, D, F, H)**.** Barrel field images were shown on both hemispheres, where the right/ipsilateral hemisphere's barrel fields (top row: A, B, C, D) were used as controls and the contralateral barrel fields (bottom row: E, F, G, H) were affected by nerve transection. Affected areas with missing barrels were marked with dashed lines. The histology of M3 was not performed, as the barrel TDI appeared normal. Scale bars are 0.2 mm.

Supplementary Figure S1. Example of the questions provided to blinded expert researchers to determine the following effects on the clarity of barrel visualisation using MRI: (1) image types; (2) full K-space and Partial Fourier-encoding acceleration; and (3) the resolutions of HARDI and stTDI.

Supplementary Figure S2. Comparison of 3D reconstruction of tangential histological slices of the barrel fields (A) after registration to stTDI map (B). Note that the intensity of the stTDI maps is displayed with inverted grey-scale contrast. Scale bars are 0.2 mm.

Supplementary Figure S3. Comparison of high-resolution 3D GE, SE and FSE at various T_2*, T_1, and T_2-weightings for barrel visualisation. Scale bar is 0.2 mm. (A) 3D Gradient echo, TR/TE=50/20ms, FA=30 degrees, 30μm isotropic, NEX=12, acquisition times = 15h 36 mins. (B) 3D Gradient echo, TR/TE=50/12ms, FA=30 degrees, 25μm isotropic, NEX=8, acquisition times = 15h 36 mins. (C) 3D Spin echo, TR/TE=400/11ms, 47μm isotropic, NEX=1, acquisition times = 4h 13 mins. (D) 3D Spin echo, TR/TE=1000/35ms, 50μm isotropic, NEX=2, acquisition times = 13h 9 mins. (E) 3D Fast spin echo, TR/TE=1000/35ms, 50μm isotropic, NEX=4, RARE factor = 4, acquisition times = 3h 14 mins.

Supplementary Figure S4. The effect of super-resolution on TDI for barrel visualisation. stTDI performed at native HARDI resolution (i.e. without super-resolution) of 100 μm (A) and 48 μm (C). stTDI reconstructed using 20 μm super-resolution from 100 μm (B) and 48 μm (D) HARDI data. Scale bar is 0.2 mm.

Supplementary Video S5. The result of 3D volumetric PMBSF delineations from stTDI map and the corresponding reconstructed histology volume. This movie is generated using the program Amira (Visualization Sciences Group).