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Comparative mouse brain tractography of diffusion magnetic resonance imaging

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ABSTRACT

Diffusion magnetic resonance imaging (dMRI) tractography can be employed to simultaneously analyze three-dimensional white matter tracts in the brain. Numerous methods have been proposed to model diffusion-weighted magnetic resonance data for tractography, and we have explored the functionality of some of these for studying white and grey matter pathways in *ex vivo* mouse brain. Using various deterministic and probabilistic algorithms across a range of regions of interest we found that probabilistic tractography provides a more robust means of visualizing both white and grey matter pathways than deterministic tractography. Importantly, we demonstrate the sensitivity of probabilistic tractography profiles to streamline number, step size, curvature, fiber orientation distribution threshold, and wholebrain versus region of interest seeding. Using anatomically well-defined corticothalamic pathways, we show how projection maps can permit the topographical assessment of probabilistic tractography. Finally, we show how different tractography approaches can impact on dMRI assessment of tract changes in a mouse deficient for the frontal cortex morphogen, fibroblast growth factor 17. In conclusion, probabilistic tractography can elucidate the phenotypes of mice with neurodegenerative or neurodevelopmental disorders in a quantitative manner.

Introduction

Understanding developmental or pathological changes in white matter tracts in animal models can lead to a better understanding of neural development and degeneration. At present, tracers or promoter-driven reporter genes such as green fluorescent protein are used as markers of brain connectivity or fiber tracts (reviewed by Kotter, 2007; Morecraft et al., 2009). These methods typically require two-dimensional analysis of a single tract and do not lend themselves to quantitative assessment of connectivity changes. Diffusion magnetic resonance imaging (dMRI) tractography can be employed to simultaneously delineate multiple cerebral white matter tracts in three dimensions and to identify possible alterations in connectivity. Diffusion MRI tractography is the process of integrating voxel-by-voxel orientations into a pathway that connects distant brain regions, and has been well described in the following reviews: Mori and van Zijl (2002), Jones (2008), Seunarine and Alexander (2009), Jones (2009) and Behrens and Jbabdi (2009).

Diffusion tractography is influenced by the anatomical preparation, data acquisition scheme, data processing (e.g. tensor, multiple tensor, non-tensor), tractography algorithm and tracking parameters. A fixed, *ex vivo*, dissected brain provides ideal tissue integrity by preserving anatomy and permitting diffusion measurements with comparatively little time constraint, thereby allowing data acquisition at the microstructural level. From this type of experiment, fractional anisotropy (FA) and mean diffusivity permit the selection of regions of interest (ROI) and the calculation of their means and standard deviation (Pierpaoli and Basser, 1996). FA provides a means of quantifying white matter differences following dMRI, but doesn't provide directional information about the white matter tracts. Directional information about fiber orientation can be generated from incorporation of the principal Eigen vector of the diffusion tensor. These data can be represented as color-coded FA maps. Tractography integrates diffusion directions to produce a path through the data, which is often represented as a streamline. The streamline propagates automatically through the vector field of a sample space along the path where the diffusion is least hindered and from this, additional metrics such as streamline length and number can be obtained (e.g. Ding et al., 2003; Correia et al., 2008). Importantly, the strategies for achieving this propagation can vary greatly according to the algorithm applied.

Diffusion MRI has been used with rodent brain previously (e.g. Mori et al., 1999; Xue et al., 1999; Song et al., 2005; Wang et al., 2006; Boretius et al., 2009; Leergaard et al., 2010). Some of these studies have used dMRI to identify and characterize neuropathologies, including three-

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dimensional structures of tract abnormalities such as Probst bundles (Ren et al., 2007). With the exception of Leergaard et al. (2010), these studies have, for the most part, used older tractography models and have not attempted to compare different approaches.

Our aim was to investigate the suitability of current tractography approaches and tractography parameters for generating streamline information from dMRI of an *ex vivo* mouse brain. We acquired dMR images using a diffusion-weighted three-dimensional spin echo sequence and a high-field strength magnet. Next, we aimed to determine the appropriate representation of the diffusion orientations and then applied deterministic and probabilistic tractography to assess the requirement of a measure of uncertainty in the streamline propagation. We compared two tractography software packages that have been used previously — TrackVis (Wang et al., 2007), which performs deterministic tractography, and FSL (Behrens et al., 2007), which performs probabilistic tractography. In addition to these established software packages, we explored tractography using the more recently developed software package MRtrix, which allows for both deterministic and probabilistic tractography (Tournier et al., 2007). Using FSL and MRtrix, we extended our study to include measures of pathways that traverse grey matter regions, such as those that connect the cortex and thalamus. Furthermore, we demonstrated that these tractography approaches could be used to identify subtle tract changes in the Fibroblast growth factor 17 knockout mouse (*Fgf17*$^{-/-}$), which has a hypoplastic frontal cortex.

Methods

Mice

All mice were housed and handled in accordance with the Institutional Animal Care and Use Committee of the University of California, San Francisco. Three adult *Fgf17*$^{-/-}$ (knockout, KO) mice and three *Fgf17*$^{+/+}$ age- and sex-matched controls were generated by mating heterozygotes (*Fgf17*$^{+/-}$) (Xu et al., 2000). Mice were anaesthetized and transcardially perfused with 0.1 M Phosphate Buffered Solution (PBS) followed by 4% paraformaldehyde (PFA). Heads were immersed in 4% PFA for a further 24 h, then washed for 24 h in PBS. At this point, heads were transported to the University of Queensland for experimentation. The brain was dissected and immersed for a further 3 days in PBS before imaging.

Diffusion MRI

Diffusion-weighted (DW) magnetic resonance images were acquired of adult mouse brains ($n=6$) immersed in Fomblin Y-VLAC oil (Y06/6 grade, Solvay, USA), using a 16.4 T vertical bore, small animal MRI system (Bruker Biospin, Rheinstetten, Germany; ParaVision v5.0) and a 15 mm linear, surface acoustic wave coil (M2M, Brisbane, Australia). Three-dimensional DW spin–echo sequences were acquired using the following parameters: TR/TE 400/22.8 ms; imaging resolution, $0.1\times0.1\times0.1$ mm (uninterpolated) and a signal average of 1. Each dataset was composed of two low diffusion-weighted images ($b=0$ s/mm^2) and thirty high diffusion-weighted ($b=5000$ s/mm^2) images with encoding gradient ($\delta/\Delta=2.5/14$ ms) vectors uniformly distributed using the electrostatic approach (Jones et al., 1999). Total imaging time was 32 h. Preliminary data, not shown here, indicated that high angular resolution diffusion imaging (HARDI) modeling of diffusion-weighted imaging in 64 or 48 directions provided no distinct advantage in tractography information, but did increase acquisition time substantially.

Tractography software

Three software packages, which are freely available to the research community, were tested. The main differences between the tractography software packages are summarized in Table 1.

Table 1
Differences between the tractography software packages.

	TrackVis	MRtrix	FSL
FOD	Qball	CSD	Bayesian and ARD
Tracking	Deterministic	Deterministic or probabilistic	Probabilistic
Seeding	Wholebrain	Wholebrain or ROI	ROI
Step size	Variable	Fixed*	Fixed*
Turning angle	Angle (step)	Curvature (step and angle)	Angle
Thresholds	Angle, mask, streamline length	FOD, streamline length	Anisotropy

ARD, automatic relevance determination. * amenable by user.

TrackVis (v0.4.4; Wang et al., 2007; www.trackvis.org) allows real-time 3D visualization and analysis of fiber tract data created by Diffusion Toolkit (Wang et al., 2007). For TrackVis, reconstruction was generated according to DTI or HARDI/Qball modeling and the Spherical Harmonic Basis method (Tuch, 2004; Hess et al., 2006). From FA and mean diffusivity generated by Diffusion Toolkit, second-order Runge-Kutta (Basser et al., 2000) and a modified version of fiber assignment by continuous tracking (referred to simply here as FACT; Mori et al., 1999; Kreher et al., 2005) were performed.

MRtrix (v0.2.7, www.nitrc.org/projects/mrtrix) uses constrained spherical deconvolution (CSD) to estimate the fiber orientation distribution (FOD; Tournier et al., 2004; Tournier et al., 2007). Tractography can be based on the diffusion tensor model (not assessed) or multi-fiber CSD, with deterministic (based on the method by Conturo et al., 1999) or probabilistic (Behrens et al., 2003; Parker et al., 2003) streamline tracking approaches. Default preprocessing parameters were used for CSD (i.e. maximum harmonic order lmax = 6).

The FMRIB Software Library (FSL, Release 4.1; Smith et al., 2004; Behrens et al., 2007; Jbabdi et al., 2007; Woolrich et al, 2009; www.fmrib.ox.ac.uk/fsl) uses a Bayesian framework with automatic relevance determination (ARD) to generate the FOD, and probabilistic tractography. In contrast with TrackVis and MRtrix, FSL does not output individual streamlines, but presents streamline-density maps or visitation maps, i.e. a map showing the number of streamlines traversing each imaging voxel.

Representation of streamlines, color maps and FA maps

Nomenclature and neuroanatomy were assigned using the Mouse Brain Atlas in Stereotaxic Coordinates (Franklin and Paxinos, 1997). For consistent representation amongst tractography programs, results are shown using an FA map overlay. Unless otherwise indicated, FA map overlays are at the level of the anterior commissure when representing horizontal or axial information, or at the midline when representing sagittal information. For coronal views, FA maps are at the level of the ROI. When describing ROIs, color-coded FA maps were used to allow visualization of the heterogeneous diffusion directions apparent within the ROI (Supp. Fig. 1). Color-coded FA maps correspond to the following orientations: red, medial–lateral; blue, dorsal–ventral; and green, rostral–caudal. Streamlines are represented by a color-coded scheme based on ROI/population. AC streamlines present in the Supplementary data are color-coded based on their orientation. Streamlines are shown in 3D while FA maps are in 2D. For the purposes of this study, the following distinction is made between seed and waypoint ROIs (see also Table 1). Seed ROIs are regions where tractography streamlines originate. In the case of TrackVis, the seed ROI is the entire brain (wholebrain). In the case of MRtrix, the seed ROI can be a selected region or wholebrain. In the case of FSL, the seed ROI is the selected region only. Waypoint ROIs are regions chosen to highlight a streamline population that has already been seeded. In TrackVis, any user-selected ROI is a waypoint (e.g. the olfactory bulb) because