LETTER

Functional Connectivity Measurement of the Brain

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Abstract — Non-invasive cognitive neuroimaging studies based on functional magnetic resonance imaging (fMRI) are of ever increasing importance for experimental and computational neurosciences. This was made possible mainly due to the availability of fast and reliable functional MRI protocols in which the brain's own hemoglobin content is used as the nature's own contrast agent. Such fMRI based cortical activation maps however, provide only the localization information of the brain, leaving its pattern of connectivity elusive. The explanatory power of the current functional MRI studies could be greatly expanded therefore, if the same MRI framework could be utilized to assess the pattern of neuronal connectivity in vivo. To this end, we have performed simultaneous fMRI and diffusion tensor imaging (DTI) based fiber reconstructions in order to elucidate the organization of large-scale neural networks governing specific cortical functions, such as the object recognition along the ventral stream. The methods developed in this study have the potential to lay foundation for *in vivo* functional neuroanatomy that can provide data fore more empirically-motivated neural network models in future.

Keywords — Functional MRI, diffusion tensor imaging, connectivity

1. Introduction

Non-invasive visualization of the cortical function using the blood oxygenation-level dependent (BOLD) functional magnetic resonance imaging (fMRI) has revolutionized cognitive neurosciences by allowing the foci of cortical "activity" to be visualized in a non-invasive manner. The BOLD contrast originates from the intravoxel magnetic field inhomogeneity induced by paramagnetic deoxyhemoglobin (deoxyHb) sequestered in red blood cells that are compartmentalized within blood vessels. The magnetic susceptibility differences between the deoxyHb-containing compartments and the surrounding space generate magnetic field gradients around the boundaries of these compartments. Therefore, perturbation of regional deoxyHb content alters the signal intensities in MR images sensitized to BOLD contrast. Such regional perturbation occurs as the result of enhanced neuronal activity and metabolism during sensory [1], motor [2], or cognitive [3] functions.

However, while functional neuroimaging based on BOLD contrast provides detailed information about the "where" of the brain's functional architecture non-invasively, such localization information alone, however, must leave pivotal questions about the brain's information processing (the "how" of the processing) unanswered, as long as the underlying pattern of neuronal connectivity cannot be mapped in an equally non-invasive manner. The explanatory power of the current functional MRI studies could be greatly expanded therefore, if the same MRI framework could be utilized to assess the pattern of neuronal connectivity *in vivo*. To this end, the newly developed magnetic resonance imaging technique known as diffusion-tensor-imaging (DTI) based on diffusion weighted imaging (DWI) has the potential to serve as such a method.

DWI MRI is one of the most widely used MRI methods for investigating the microscopic structure of water-containing material in general, and living tissue in particular [4]. As suggested by Stjeskal and Tanner [5], the MR image is sensitized to diffusion in a given direction using a couple of temporally separated magnetic field gradients in the desired direction. The first gradient labels the spin phase of the water molecule protons along the gradient direction, while the second gradient rephrases the spin phase. If no diffusion has occurred during the time period between the two gradients, the initial phase is fully restored, while the initial phase is not fully recovered if water molecule displacement has taken place. In such case the resulting image intensity is attenuated.

The amount of image attenuation, or "diffusion weighting", is given by the relation $S(b) = S_0 \exp(-b_i D_i)$ where S is the signal intensity and S_0 is the signal intensity without diffusion weighting. D_i is the diffusion coefficient of water in the direction on which of the magnetic field gradient was applied, and b is given by: $b = \gamma^2 g^2 \delta^2 (\Delta - \delta/3)$ in a spin-echo experiment, where g is the gradient strength, γ is the gyromagnetic ratio of protons, δ is the gradient duration time and Δ is the gradient separation time.

Because the parameters g, γ, δ, and Δ are all known, from the amount of signal decrease (S/S_0), diffusion constants at each voxel can be derived. Such measurements have revealed that diffusion of brain water has strong directionality (anisotropy), which is attributed to the existence of natural boundaries, such as axons and/or myelination. The properties of such water diffusion can be expressed as an ellipsoid [6]. Such "diffusion ellipsoids" can be characterized by six parameters: diffusion constants along the longest, middle, and shortest axes (λ_1, λ_2, and λ_3, called *principal axes)* and the direction of the three principal axes. Once the diffusion ellipsoid is fully characterized at each pixel of the brain images, local fiber structure can be derived. For example, if $\lambda_1 >> \lambda_2 \geq \lambda_3$ (diffusion is *anisotropic*), it suggests the existence of dense and aligned fibers within each pixel, whereas isotropic diffusion ($\lambda_1 \approx \lambda_2 \approx \lambda_3$) suggests sparse or unaligned fibers.

In the past few years, numerous other DTI fiber reconstruction algorithms have been proposed that reveal spectacular images of axonal connectivity patterns *in vivo* both in humans [7-9], rodents [6, 10], and cats [11]. The differences in detailed fiber reconstruction algorithms notwithstanding, all DTI based fiber reconstructions are faced with a fundamental and peculiar problem: Unlike conventional neurotracing techniques (e.g. using DiI, HRP, biocytin etc.; see Discussion) - each DTI experiment has the potential to provide the complete set of connectivity across all imaged voxels. Consequently, if the fiber reconstructions are not limited to particular regions of interest (ROI), the resulting pattern of fiber connections would be rendered useless. A key in making DTI an outstanding technique for cognitive neurosciences is therefore to develop selection criteria to determine the seeding region of interest (ROI) for DTI fiber tracing. To this end, in the majority of fiber-tracking algorithms, tracking starts at a user-defined seeding point or region of interest. Such "seeding points" are selected either based on the quality of the underlying DWI, or based on *a priori* anatomical criteria that are known from postmortem studies. Such anatomically-motivated tracking strategies are of greatest importance for testing for anatomical irregularities *in vivo*. An alternative way of DTI fiber reconstruction is to use the foci of functional activity - such as obtained with BOLD contrast - as the "initial" and "termination" ROIs. This is a more natural choice for most questions in cognitive neurosciences, as the main interest here is to elucidate the pattern of neuronal circuitry underlying the observed functional activation for a particular task. In this study we have performed simultaneous fMRI and diffusion tensor imaging (DTI) based fiber reconstructions in order to elucidate the organization of large-scale neural networks governing specific cortical functions, such as the object recognition along the ventral stream.

2. Materials and Methods

Subject preparation: All studies were performed with the approval of the Institutional review Board (IRB) of the University of Minnesota Medical School and Boston University School of Medicine. Following the proper instruction of the subjects, the subjects were asked to lay on the MRI table, inside the magnet and view visual stimuli. Paddings and foams were used to maintain the subject's head in a stable position. Earplugs and headphones were employed to reduce the noise due to the switching gradients.

Visual stimulation: Visual stimuli were generated on a PC using custom written MATLAB (The Mathworks Inc., Natick, MA, USA) software utilizing functions provided by PsychToolbox[12]. Stimuli were presented binocularly with a video projector on a rear projection screen. Conventional checkerboard stimuli that consisted of four triangular wedges for the upper/lower and left/right visual field, and four segmented expanding rings for foveal representation were used for mapping retinotopic areas. Localizer stimuli known to activate the respective areas within the human ventral stream were used to identify the FFA (conventional/scrambled faces)[13], PPA (buildings and scenes), and LOC (set of complex objects). Within each all-novel epoch, subjects saw four categories of pictures that each contained thirty different photographs. Within multiple-repeat epochs (4 repetitions), subjects saw different photographs from the same category. A localizer was used to identify hMT+ (motion) as a part of human dorsal stream.

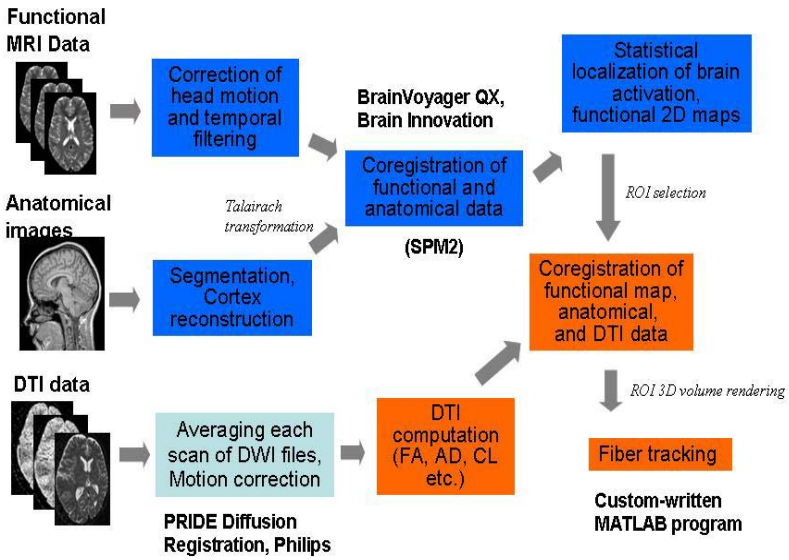

Figure 1. Data analyses strategy for combined fMRI/DTI experiment.

MRI acquisition: High resolution fMRI and T1-weighted anatomical images were obtained at 3 Tesla (Siemens Trio or Philips Intera). The Imaging parameters for the Siemens scanners were: T1 MPRAGE (non-selective IR), NrOfSlices: 144, SliceThickness: 1 mm, FoV: 256 mm x 256 mm, Matrix: 256 x 256, TR: 2100 ms, TE: 3.93 ms, TI: 1100 ms, Flip angle: 15 degrees, 1 NEX; fMRI: Gradient echo EPI: NrOfSlices: 30**,** SliceThickness: 2 mm**,** FoV: 256 mm x 256 mm, Matrix: 128 x 128**,** NrOfVolumes: 132, TR: 3000 ms, TE: 40 ms. Parameters for the Philips scanner: Anatomy: T1 MPRAGE (NS-IR): NrOfSlices: 144, Slice Thickness: 1 mm, FoV: 230 mm x 230 mm, Matrix: 256 x 256, TR: 2100 ms, TE: 4.6 ms, TI: 1100 ms, Flip angle: 8 degrees, 1 NEX; fMRI: Gradient echo EP: NrOfSlices: 30, SliceThickness: 2 mm, FoV: 230 mm x 230 mm, Matrix: 128 x 128, NrOfVolumes: 132, TR: 3000 ms, TE: 40 ms.

Diffusion-weighted MRI: Conventional methods for diffusion-weighted imaging were used in order to calculate the voxel-based diffusion tensors. Diffusion imaging parameters for the Siemens scanner were: DTI: Spin echo EPI, NrOfSlices: 64, Slice Thickness: 2 mm, FoV: 256 mm x 256 mm, Matrix: 128 x 128, NrOfDirections: 12, TR: 11500 ms, TE: 111 ms, 3 NEX. Parameters for the Philips scanner: DTI: Spin echo EPI, NrOfSlices: 73, SliceThickness: 1.5 mm, FoV: 230 mm x 230 mm, Matrix: 256 x 256, NrOfDirections: 15, TR: 10646 ms, TE: 91 ms, 1 NEX

Diffusion Tensor calculation: Diffusion tensor elements (e.g. for 6 gradient direction encodings) were calculated using the following set of equations. Here, the letters X, Y, Z in parentheses denote the directions along which the gradients were applied, and the subscript of D denotes the respective element of the diffusion tensor $\overline{\overline{D}}$, S_0 is the image without diffusion weighting and S_{xyz} are the diffusion weighted images where the gradients are applied along the direction defined in the subscript:

$$\text{(X,Y,0):}\ (D_{xx} + D_{yy} + 2D_{xy}) = -\frac{\ln S_{110} / S_0}{b} \tag{1-1}$$

$$\text{(X,-Y,0):}\ (D_{xx} + D_{yy} - 2D_{xy}) = -\frac{\ln S_{1-10} / S_0}{b} \tag{1-2}$$

$$\text{(X,0,Z):}\ (D_{xx} + D_{zz} + 2D_{xz}) = -\frac{\ln S_{101} / S_0}{b} \tag{1-3}$$

$$\text{(-X,0,Z):}\ (D_{xx} + D_{zz} - 2D_{xz}) = -\frac{\ln S_{-101} / S_0}{b} \tag{1-4}$$

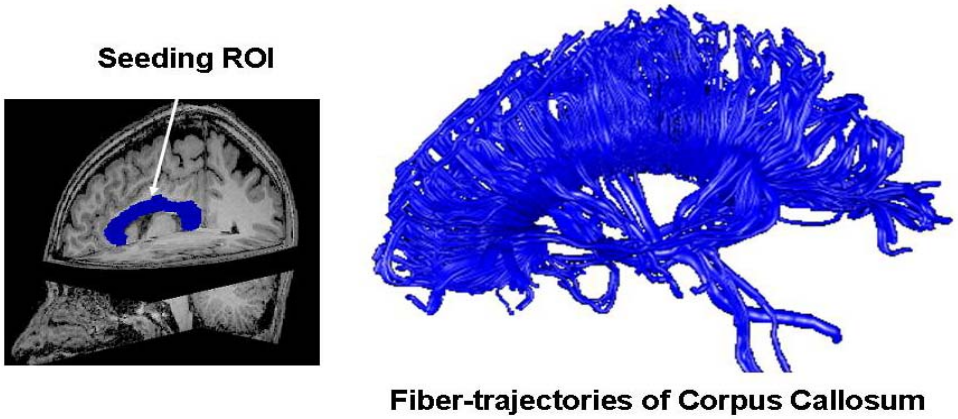

Figure 2. *In vivo* High-resolution Diffusion Tensor Imaging (DTI) of the human corpus callosum.

$$(0,Y,Z):\ (D_{yy} + D_{zz} + 2D_{yz}) = -\frac{\ln S_{011} / S_0}{b} \tag{1-5}$$

$$(0,-Y,-Z):\ (D_{yy} + D_{zz} - 2D_{yz}) = -\frac{\ln S_{01-1} / S_0}{b} \tag{1-6}$$

From the equations (1-1)-(1-6), we can form a tensor matrix [20]:

$$\overline{\overline{D}} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix} \tag{2}$$

Since the tensor matrix $\overline{\overline{D}}$ is symmetric along the diagonal, the eigenvalues and eigenvectors can be obtained by diagonalizing the matrix using the Jacobi transformation. The resulting eigenvalues $\overline{\overline{\Lambda}} = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix}$ and corresponding eigenvectors $\overline{\overline{P}} = \left[\overrightarrow{p1} \quad \overrightarrow{p2} \quad \overrightarrow{p3}\right]$ can then be used to describe the directionality of water diffusion within a given voxel. There are several different methods how this can be achieved. For the present study, the directionality of water diffusion was estimated by computing the fractional anisotropy (FA) on a voxel-by-voxel basis [14].

$$FA = \frac{1}{\sqrt{2}} \sqrt{\frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}{\lambda_1{}^2 + \lambda_2{}^2 + \lambda_3{}^2}} \tag{3}$$

The fractional anisotropy (FA) is a measure of the directionality of the water diffusion within a given voxel.

DTI fiber reconstruction: Based on the diffusion tensors, a fiber-tracking algorithm was applied to the data using custom-written C++ or Matlab (Mathworks, MA) codes. The algorithm of choice was one based on Frenet equation, similar to Basser's algorithm [8], additionally modified to include a criterion for the vector field interpolation, in order to remove noise and background effects (see [15] for details). The fractional anisotropy (FA) threshold for stopping the tracking procedure was set to 0.2. ROIs for seeding the tracking algorithm were chosen either manually, or based on BOLD fMRI activation foci. In either case, the seeding ROIs were chosen close to the fiber termini, in order to ensure that they included white matter areas. Figure 1 displays the overall sequence of data analyses steps from initial MRI acquisition to multimodal fusion of fMRI data with DTI fiber tractography.

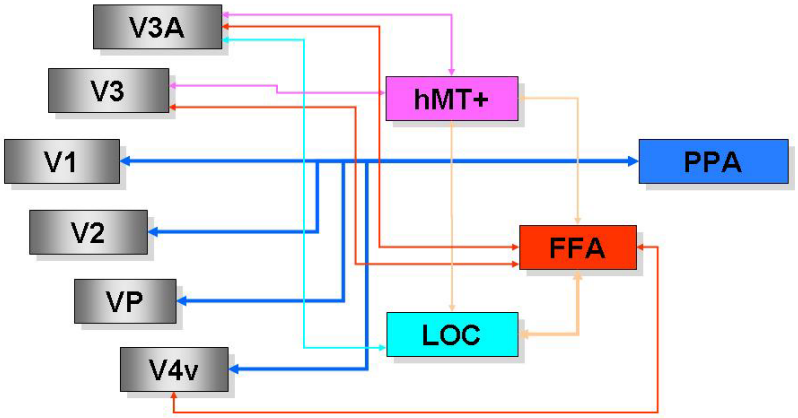

Figure 3. *In vivo* circuitry of the human occipito-temporal cortex.

3. Results

Figure 2 displays the results of high-resolution DTI studies in human corpus callosum. The shape, density, and the overall organization of these DTI based fiber trajectories resemble what is known from postmortem neuroanatomy of the human brain. Similar and numerous other DTI fiber tractography results were obtained in recent years by a large number of investigators, suggesting the principal usability of DTI based fiber reconstructions. However, it is important to note that the ultimate veracity of DTI fiber tractography remains elusive. This is because DTI is fundamentally prone to erroneous fiber reconstructions due to poor signal-to-noise ratio, non-Gaussian diffusion, fiber crossings, and other uncertainties. Some of these uncertainties can be mitigated through the use of high-angular diffusion techniques such as diffusion spectrum imaging (DSI) and q-ball techniques. An alternative approach is to calculate the probability for the fiber presence, thus yielding a probabilistic map for cortical connectivities.

Finally, Figure 4 demonstrates the possibility of compiling a comprehensive *in vivo* circuitry diagram of the human brain if DTI tractography is combined with conventional functional MRI scans. In this case, we were able to reconstruct the occipito-temporal visual pathways of the human brain.

4. Discussions and Conclusions

The results of our study suggest that high resolution BOLD MRI and Diffusion Tensor Imaging (DTI) can be obtained from the same cortical tissue *in vivo* at 3Tesla magnetic fields. Furthermore, in our study, the foci of fMRI activation were successfully utilized as seeding points for 3D DTI fiber reconstruction algorithms, thus providing the map of the circuitry between neuronal populations participating for a common cortical information processing across the human occipito-temporal cortex. Traditionally, connectivity between individual neurons or groups of neurons have been studied using a variety of techniques. These include *post mortem* methods, such as dissection of white matter, strychnine neuronography [16], and the Nauta [17] methods of tracing degeneration after localized lesions. More recently, implantation of carbocyanine dyes, such as DiI and DiA [18], has replaced the older degeneration methods. However, besides the long duration needed for passive labeling of axonal fibers (often several months), *post mortem* methods inherently fail to yield a correlation to the foci of functional activation. There are other modern *in vivo* labeling methods that can be used to track patterns of neuronal connectivity include trans-neuronal markers, such as horseradish peroxidase (HRP), rhodamine- and fluorescein-conjugated latex microspheres [19], biocytin [20], and biotinylated dextran amine (BDA). However, all current postmortem and *in vivo* tracing techniques suffer from common limitations: a) an extremely low number of labeled tracts can be identified; b) short tracing distance (across 1-3 synapses); c) the need for invasive injection of the tracer, and most importantly, d) the need to sacrifice the animal before the labeling pattern can be visualized. Such limitations rule out the use of these methods for human and/or longitudinal studies.

The BOLD-based DTI fiber reconstruction method described in this study allows the local orientation of fiber bundles in the white matter to be determined in a non-invasive manner, thus enabling *in vivo* neuroanatomy in both animals and humans. If the full potential of fMRI based DTI for tracking fiber pathways non-invasively

in human and animal brains can be realized, the impact on neuroscience will be substantial. Knowing which functional areas are connected to which, and in what manner, can provide vital constraints on high-level models of global cortical organization.

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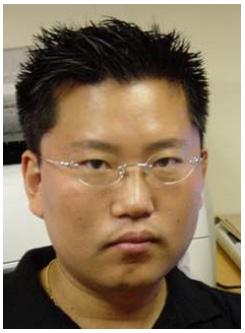

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