DTI/fMRI: Integration/Synergy

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Introduction

The rapid progress of blood oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) in recent years[1-3] has raised the hope that the functional architecture of the human brain can be studied directly in a non-invasive manner. The BOLD technique is based on the use of deoxyhemoglobin as nature's own intravascular paramagnetic contrast agent[4-6]. When placed in a magnetic field, deoxyhemoglobin alters the magnetic field in its vicinity, particularly when it is compartmentalized as it is within red blood cells and vasculature. Increases in neuronal and metabolic activity of the brain results in a *reduction* of the relative deoxyhemoglobin concentration due to an increase of fresh oxyhemoglobin. Consequently, in conventional BOLD fMRI, brain "activity" can be measured as an *increase* in T_2 or T_2^* weighted MR signals[1-3]. Since its introduction about 10 years ago, BOLD fMRI was successfully applied – among numerous other examples - to precisely localize the cognitive[7], motor[8], and perceptual[9-11] function of the human *cortex cerebri*. The explanatory utility of BOLD fMRI has been further strengthened in recent years through the introduction of high (~3T) and ultra-high (~7T) MRI scanners[12]. Stronger magnetic field not only increases the fMRI signal *per se*, but in addition it will specifically enhance the signal components originating from parenchymal capillary tissue, thus enhancing the spatial specificity of BOLD fMRI.

While the BOLD fMRI technique provides detailed information about the spatial location of the functionally active cortical areas, the question of functional interdependency between the cortical areas remains elusive. This is of particular importance for higher cortical functions, as they require a well-coordinated balancing of cortical computations across multiple brain areas. This is most evident in the mammalian visual system. Here, similar to other primates, human visual areas are clustered along two "streams" diverging from the occipital pole: the ventro-temporal "what or perception" stream and the dorsal "where or action" stream[13, 14]. For example, a region in the lateral occipital cortex (LOC) extending anteriorly into the temporal cortex responds strongly to a variety of complex shaped objects such as polygonal figures, chairs, and gloves, etc.[15, 16]. On the other hand, in the so called fusiform face area (FFA)[17] located within the fusiform gyrus, cells are tuned to faces and facial stimuli in a way comparable to the receptive field properties of face-selective neurons in primate inferotemporal cortex (IT)[18, 19]. Further down the temporal cortex, in the so called parahippocampal place area (PPA)[20], maximum functional response can be obtained using scenic or place type of stimuli.

In the present study, we used the newly developed diffusion-tensor-imaging (DTI) technique to elucidate the pattern of connections between the distinct functional modules of the human occipito-ventral visual stream. Diffusion Tensor Imaging (DTI) is a powerful MRI technique that enables us to translate the self-diffusion, or microscopic motion of water molecules in tissue into a MRI measure of tissue integrity and structure. Specifically, in white matter, water self diffusion is restricted mostly by the intracellular axonal space, and by the interstitial, extracellular space among the well-packed axons in the fiber tract. By taking several diffusion weighted images in several dimensions, one can reconstruct the so-called diffusion tensor for each image unit, or pixel. The diffusion tensor gives a three dimensional representation of the preferred direction of diffusion, in the shape of the 3D ellipsoid. The main axis of the ellipsoid gives the "preferred" direction of diffusion, typically parallel to the axonal tract, and the two minor axes perpendicular to the main direction typically provide a measure to "lateral" diffusion, or the ability of water molecules to move in a direction perpendicular to the main direction. Recently, DTI techniques in combination with a variety of 3-D fiber reconstruction algorithm was used to generate spectacular images of the axonal connectivity pattern *in vivo* both in humans[21-23], rodents[24], and recently also in cats[25]. The differences in detailed fiber

reconstruction algorithms notwithstanding, a key in making DTI into an outstanding tool for cognitive neurosciences is to develop selection criteria to determine the seeding region of interest (ROI) for DTI fiber tracing. 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*. However, in normal subjects, strictly anatomically defined DTI fiber reconstructions have the tendency to simply re-validate what has been already known from conventional anatomical and histological techniques, thus resulting in partially tautological statements. An alternative way of DTI fiber reconstruction therefore 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.

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[26]. 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)[27], 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.

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, SliceThickness: 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, SliceThickness: 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

Data analysis: Functional imaging scans were used to localize areas hMT+, LOC, FFA, and PPA, as well as retinotopic areas (V1, V2, V3, V3A, VP and V4v). fMRI data were analyzed using BrainVoyager (Brain Innovation, Maastricht, Netherlands). Each area was segmented by mapping borders in the flattened representation of cortex, and reconstructed as 3D volume-rendered ROIs. ROIs were then imported into a custom-written DTI reconstruction software. Diffusion tensors, fractional anisotropy (FA), and fiber tracts were calculated using custom-written MATLAB (The

Mathworks Inc., Natick, MA, USA) software. Standard methods for FA exclusion and tracking algorithms were used[22]; i.e. minimum FA of 0.2 and 60 degrees of Maximum angle. Since fMRI activation is limited to the gray/white matter border, we chose to include at least one more voxel (beyond the gray/white matter boundary) into our seeding ROI. To investigate the connectivity pattern, tracing was performed between two selected ROIs (i.e. V1 & PPA pair, V2 & FFA pair etc.) for each individual area. Subsequently, corresponding fMRI and DTI tracing data were superimposed on three-dimensional anatomical images for visualization.For high-order non-retinotopic visual areas, the respective "localizer" stimuli were used. Talairach coordinates of the investigated areas were found to be in well agreement with previous published results; hMT+: (46, -58, 4), LOC: (46, -63, 8), FFA: (36, -48, -16), and PPA: (28, -39, -6).

Results and Discussions

Following the initial localization, voxels from retinotopic and non-retinotopic iso-functional areas were used as "seeding" points for DTI based fiber reconstructions. Our data demonstrate – from the same subject – the pattern of connections between V1/V2 <--> PPA and V3 <--> FFA, respectively. Overall, of the functionally defined areas tested (FFA, PPA, LOC, and hMT), PPA was found to be the most highly connected to the retinotopically defined visual areas. Strong connections were found between the PPA and areas VP and V4v. In addition, we also found pronounced connections between PPA and visual areas V1 and V2. In contrast, a significant connection between primary visual areas (V1/V2) and FFA was observed in only one subject (out of 4 subjects used for this type of analysis). On the other hand, connections to/from FFA originated (or ended) mostly in V3, V4v and V3A (observed in 3 out of 4 subjects analysis for this study). The most pronounced connection for area LOC was with area V3A (3 out of 4). hMT was also found to have significant connections to V3A in addition to area V3.

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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 3 Tesla 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 axonal circuitry between neuronal populations participating in occipito-ventral visual information processing. The results of our preliminary study suggest that the functional organization of the human occipito-ventral stream is governed by a distinct pattern of inter-areal connectivities. the areas hMT+, LOC, and FFA are tightly interconnected, thus forming a loop for visual information processing. The principal connections to/from this ventral loop are provided by areas V3 and V3A, while the influence of the primary visual areas V1 and V2 to/from this processing loop is limited. The observed connections between V3/V3A and FFA are consistent with the hierarchical pattern of visual area known from macaques[28]. Consistent with classical tractography work done for the visual_system of rhesus macaques, our fMRI/DTI data suggest a linear connectivity relationship between association visual cortex of the occipital lobe and association cortex of the temporal lobe.

Overall, our combined fMRI/DTI studies resulted in occipio-ventral stream connectivity pattern that is highly specific and comparable to homologous data obtained in macaques using well-established neuroanatomical techniques. However, before this new technique can be utilized for addressing clinical and basic neuroscience questions *de* novo, major interpretative issues will have to be addressed. Some of the critical issues are:

Can we trust DTI based fiber reconstructions? Despite intense research, the structural correlate of DTI remains elusive. For example, the precise contribution of the underlying fiber density and myelination on the anisotropy index has not been completely understood. Thus, it is not clear to what degree the results of DTI correspond to actual density and orientation of the local axonal fiber bundles. It is also important to understand how white matter is, in general, organized. How often do fiber pathways cross, and when they do, what happens? Can fibers change direction sharply in deep white matter? How often do individual fibers branch? When axons descend from columns in grey matter into white matter what route do they take? Do they remain together or do they intermingle with fibers from more distant regions of cortex?

Selection criteria for DTI data: In addition to the reliability and validity problems related to DTI methodologies *per* se, the combination of DTI with functional MRI scans raises a set of unique challenges and problems. The relevant issues are: a) How do we extract compact seeding ROIs based on "patchy" BOLD functional activity? What is the appropriate statistical threshold for this? Should all fMRI voxels (above certain threshold) be treated as homologous DTI seeding ROIs regardless of their p-values and/or cross correlation coefficients? b) Areas of high BOLD activity are located within the cortical gray matter. The cortical gray matter, however, is characterized by low fractional anisotrophy (because of the presence of multi-directional fibers/fiber bundles within the imaging voxels in the gray matter). Therefore, most reconstructed fibers will terminate at the gray/white matter boundary. If seeding ROIs were placed exclusively within the fMRI voxels, conventional DTI algorithms will result in zero or low number of reconstructed fibers. We will therefore need to develop automatic "search" algorithm (this is currently done manually) for the closest fiber termination points which have to be included as part of the seeding ROI. c) We will need to address the question how the experimentally acquired voxel size should be related to the density of the seeding points. For example, a given $1x1x1mm^3$ voxel in the BOLD fMRI study may contain hundreds of thousands of physical axonal fibers. For the subsequent DTI fiber reconstruction, should the same voxel be then treated as ONE seeding point? Or should the imaging voxel be "regrided" into a multitude of sub-seeding points?

In summary, 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 an absolutely non-invasive manner, thus enabling *in vivo* neuroanatomy in both animals and humans. The methods developed in this study has the potential to lay foundation for *in vivo* neuroanatomy and the ability for non-invasive longitudinal studies of brain development.

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