DIFFUSE DISCONNECTIVITY IN TBI: A RESTING STATE FMRI AND DTI STUDY

Abstract
Diffuse axonal injury is a common pathological consequence of Traumatic Brain Injury (TBI). Diffusion Tensor Imaging is an ideal technique to study white matter integrity using the Fractional Anisotropy (FA) index which is a measure of axonal integrity and coherence. There have been several reports showing reduced FA in individuals with TBI, which suggest demyelination or reduced fiber density in white matter tracts secondary to injury. Individuals with TBI are usually diagnosed with cognitive deficits such as reduced attention span, memory and executive function. In this study we sought to investigate correlations between brain functional networks, white matter integrity, and TBI severity in individuals with TBI ranging from mild to severe. A resting state functional magnetic resonance imaging protocol was used to study the default mode network in subjects at rest. FA values were decreased throughout all white matter tracts in the mild to severe TBI subjects. FA values were also negatively correlated with TBI injury severity ratings. The default mode network showed several brain regions in which connectivity measures were higher among individuals with TBI relative to control subjects. These findings suggest that, subsequent to TBI, the brain may undergo adaptation responses at the cellular level to compensate for functional impairment due to axonal injury.

Keywords
- Traumatic Brain Injury (TBI) • Functional magnetic resonance imaging (fMRI) • DTI • Cognitive Function

List of Abbreviations
ACC - Anterior Cingulate Cortex
BDI - Beck Depression Inventory
BISQ - Brain Injury Screening Questionnaire
CC - Corpus Callosum
DAI - Diffusion Axonal Injuries
DMN - Default Mode Network
DTI - Diffusion Tensor Imaging
FA - Fractional Anisotropy
fMRI - Functional Magnetic Resonance Imaging
ICA - Independent Component Analysis
ICBM - International Consortium for Brain Mapping
MCI - Mild Cognitive Impairment
MD - Mean Diffusivity Maps
MNI - Montreal Neurological Institute
PC - Parietal Cortices
PCC - Posterior Cingulate Cortex
TBI - Traumatic Brain Injury
TBSS - Tract Based Spatial Statistics
TFCE - Threshold-Free Cluster Enhancement

1. Introduction
The pathophysiology of Traumatic Brain Injury (TBI) is complex and not well understood. Imaging research has shown that the primary mechanisms of injury are bleeding and diffuse axonal injuries (DAI) [1-3]. Cognitive impairment including diminished attention, memory and executive function are observed in individuals with TBI with injuries at all severity levels [4-6]. Routine radiologic exams of people who suffered mild to moderate TBI do not always show positive readings that correlate with their cognitive impairments [7,8]. Abnormal white matter as well as gray matter has also been correlated with TBI severity. Using Diffusion Tensor Imaging (DTI), DAI have been shown as multifocal hyperintensities on T2 weighted sequences as well as reduced Fractional Anisotropy (FA), a measure of white matter integrity [9]. Cognitive impairments have been correlated with reduced FA in white matter in several brain regions [10,11].

Functional imaging studies based on blood flow measures [12] have shown aberrant activation patterns in the attention and memory circuitry in individuals with TBI, with TBI subjects showing higher activity compared to control subjects [13-16]. These functional studies are consistent with the notion that reduced efficiency consequent to brain injury results in a need to recruit additional neuronal resources to accomplish a task [17,18]. One limitation of these task driven functional imaging studies is that differences in task performance among individuals with TBI and controls may confound the findings. Since most TBI subjects suffer from cognitive impairments, it can be difficult to determine whether imaging findings are attributable to differences in cognitive abilities, structural changes, or true functional differences. Resting state functional magnetic resonance imaging (fMRI) is an alternative functional imaging technique that circumvents this potential confound, as resting state sequences do not require the subject to...
perform any cognitive task while undergoing the fMRI scan [19-21]. To date there have been limited resting state studies in TBI, limited to case studies [22] or to a very small number of subjects [23].

Results from most of the imaging studies are consistent in demonstrating a global disconnect in the brains of TBI subjects. Given that TBI subjects have white matter abnormality and functional differences in brain activation studies, we sought to investigate gender effects in the correlates between structural connectivity and functional connectivity using DTI and resting state fMRI. In this study we have limited our TBI patient selection to those with mild to severe TBI and limited or no visible anatomical lesions based on assessments using conventional imaging protocols.

2. Experimental Procedures

2.1 Subjects

Twelve subjects diagnosed with mild to severe TBI and eleven age- and gender-matched controls were recruited for this imaging study. Mean age was 39.82 (Controls=40.1, TBI=39.6). All participants were assessed using the Brain Injury Screening Questionnaire (BISQ, Brain Injury Research Center of Mount Sinai School of Medicine 1997, 2001), the Beck Depression Inventory (BDI), and the Beck Anxiety Questionnaire (BAI). The BISQ is a self-report measure of anxiety. In injury severity was classified using a 7-point scale ranging from 1 (No loss of consciousness, no confusion (i.e., no TBI)) to 7 (Loss of consciousness greater than 4 weeks in duration).

2.2 Imaging

All images were acquired on a Philips 3.0 T Achieva (Best, The Netherlands) scanner. A proton density/T2-weighted, dual echo imaging sequence was used to screen for incidental pathology (TR = 2500 ms, TE = 10, 80 ms, FOV = 23.0 cm, FA = 90°, 36 axial slices, thickness = 3 mm, skip = 1 mm, Matrix size = 400 x 312). One fMRI rest scan was acquired using a field echo EPI sequence with the following parameters: TR = 2000 ms, TE = 27 ms, FOV = 21.0 cm, FA = 90°, 38 slices, skip = 0.8 mm, Matrix size = 88 x 86, 120 dynamics. A diffusion-weighted spin echo sequence was used to acquire the DTI image (TR = 5682 ms, TE = 70, FOV = 21.0 cm, FA = 90°, 54 axial slices, thickness = 2.5 mm, no skip, matrix size = 104 x 106, b-factor = 1200 s/mm², 32 gradient directions plus b0, 2 averages). A high resolution 3D T1-weighted structural image with good grey/white matter differentiation was acquired using a fast-field echo sequence for coregistration and normalization purposes (TR = 7.5 ms, TE = 3.4 ms, FOV = 22.0 cm, FA = 8°, 172 sagittal slices, thickness = 1 mm, no skip, Matrix size = 220 x 204 x 172). For the resting state fMRI we used a GE-EPI sequence with TR=2000ms, TE=27ms, 38 slices, thickness = 2.5mm, skip =0.8mm, FOV 20.8cm, Matrix size = 88x86, 120 time points for a total of 4 mins.

2.3 Data Analysis

2.3.1 Diffusion Tensor Imaging

Diffusion Tensor Images were eddy-current-corrected. FA and mean diffusivity maps (MD) were calculated using the FSL comprehensive library of analysis tools for brain imaging data (www.fmrib.ox.ac.uk/fsl). Exploratory whole brain group comparisons of the diffusion parameters were performed. First, FA images were spatially normalized to the International Consortium for Brain Mapping (ICBM) brain template using Tract Based Spatial Statistics (TBSS) [24]. The procedure involves a skeletonization of the FA images to obtain centers of white matter tracts. Voxel-wise statistics are performed only on the white matter skeleton in order to reduce the chance of type I errors due to imperfections in normalization. The parameters used to warp the FA images to the ICBM template and the white matter skeleton were applied to the MD images for statistical comparison. Randomize is an FSL routine for permutation based inference testing that was used for voxel-wise general linear modeling to test for group differences in FA and MD (separately). Clusters were identified using the TFCE (Threshold-Free Cluster Enhancement) that is optimized for permutation based inference testing of skeletonized images [25]. FA values of whole brain and several individual tracts were also extracted for further statistical analysis using Statistica V9 (Statsoft Inc, Tulsa, OK). In addition, the corpus callosum (CC) as visualized on a midsagittal slice was analyzed separately. In-house software developed in Matlab 2009 was used for tracing of the CC. Tracing was performed on an edge enhanced FA image using a Sobel filter. The average FA of the CC was calculated. T-tests were used to determine group differences in FA and self-reported depression, and correlational analyses were used to assess relationships between injury severity and outcomes among individuals with TBI.

2.4 Resting State Analysis

Preprocessing of the functional images was performed in FSL and included motion correction (MCFLIRT), coregistration to the high resolution T1 images (FLIRT) and non-linear registration to the standard Montreal Neurological Institute (MNI) template (FNIRT). Independent component analysis (ICA) was used to identify 18 unique networks of resting state activity using MELODIC [26] as implemented in FSL. The default mode network (DMN) consisting of the Anterior and Posterior Cingulate Cortex (ACC & PCC) and the bilateral parietal cortices (PC) has been consistently reported before by other studies [19-21]. The DMN was identified for each subject and z-statistic images were extracted and entered higher-level analyses. General linear modeling and permutations-based inference testing (RANDOMISE) were used to test for group differences and symptom correlates of the default mode network.

Table 1. Neuropsych assessment for mild to moderate TBI subjects.

<table>
<thead>
<tr>
<th>Clinical Assessment</th>
<th>Mean Control</th>
<th>Mean TBI</th>
<th>t-value</th>
<th>df</th>
<th>p</th>
<th>n controls</th>
<th>n TBI</th>
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<tbody>
<tr>
<td>BDI</td>
<td>1.60</td>
<td>12.67</td>
<td>-3.42</td>
<td>20</td>
<td>0.0027</td>
<td>10</td>
<td>12</td>
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<tr>
<td>BAI</td>
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<td>6.5</td>
<td>-2.69</td>
<td>21</td>
<td>0.0137</td>
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<td>12</td>
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<tr>
<td>Severity</td>
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<td>4.54</td>
<td>-</td>
<td></td>
<td>-</td>
<td>11</td>
<td>12</td>
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