Examination of Functional Reorganization in Multiple Sclerosis using fMRI-Guided Magnetic Resonance Spectroscopy: A Pilot Study

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Abstract

Introduction: Compared to healthy controls (HCs), individuals with multiple sclerosis (MS) show aberrant brain activation patterns during performance of certain tasks. Such patterns of activity have been interpreted as restructuring of functional connections, i.e. the brain’s ability to change neural networks in response to pathology. However, the relationship between neural damage related to MS and abnormal brain activation is not well understood. Here, we utilized proton magnetic resonance spectroscopy 1H-MRS, a technique sensitive to underlying pathological substrates, to examine neurometabolite levels in the brain of MS individuals in conjunction with fMRI in order to better understand the relationship between neuropathology and brain activity in MS.

Methods: Neurometabolite levels in pre-selected regions were correlated with brain activity measured with fMRI during a processing speed task in a small sample of 8 individuals with MS and 9 HCs.

Results: A positive correlation between brain activity and the N-acetylaspartate (NAA) and choline (Cho) levels was noted in specific regions, indicative of neuronal injury and increased membrane turnover, respectively.

Conclusions: Combining fMRI and MRS might be a useful approach for predicting brain pathology and its associated effects on functional brain activation in individuals with MS.

Introduction

Multiple studies examining both cognitive and motor impairments in MS report that individuals with MS show aberrant brain activation compared to healthy adults [1,2], including recruitment of additional brain regions [3], as well as decreased activation compared to controls [2,4]. Such patterns of brain activity are often interpreted as restructuring of functional connections [5-8]. That is, due to neuropathology caused by MS, additional neural networks are recruited as a result of increased task demands or reduced cerebral resources. However, the relationship between neuropathology detected by conventional MRI and brain activation detected by fMRI has been difficult to interpret. This difficulty may be due to the limitations of conventional MRI in providing information about specific types of pathology in MS, such as damage to normal-appearing white matter (NAWM). NAWM damage has been hypothesized to be the most closely related to irreversible disability [9-11] and is likely to contribute to functional activation changes. In order to better interpret functional changes observed with fMRI, it is essential to examine not only structural but metabolic damage that has occurred as a result of MS. Proton Magnetic Resonance Spectroscopy (1H-MRS) allows the examination of biochemical changes in the normal appearing tissue (NAA), a marker for neuronal integrity, are correlated with functional cerebral changes during motor tasks (aberrant brain activity patterns in MS) compared to HC. For example, Reddy et al. found that during a motor task, activation of the ipsilateral sensorimotor cortex was increased in individuals with MS relative to HCs, and a strong negative correlation was observed between NAA levels and increased brain activity.
activity in the ipsilateral sensorimotor cortex [19]. Similarly, Rocca et al. found that during a repetitive flexion-extension task, individuals with MS showed significantly more activity in the contralateral primary and secondary somatosensory cortex and inferior frontal gyrus compared to HCs [17]. Activation in the contralateral primary somatosensory cortex was negatively correlated with whole brain NAA levels.

The current study is the first to examine the relationship between neurometabolite levels in MS-affected brain tissue and task-related changes in brain activity (assessed with fMRI) during a cognitive task. Specifically, in the current study we examined the relationship between neurometabolite levels and BOLD activity during performance of a visual processing speed task, since processing speed deficits are reported to be the most significant and prevalent cognitive impairment in MS [20]. In accordance with previous motor studies [19,21], we predicted that NAA levels (indicating increased neuropathology) will be correlated with BOLD activity in brain regions that are engaged during the processing speed task.

Additionally, this study will examine the relationship between Choline (Cho) and brain activity. Elevated Cho levels are indicative of demyelination/remyelination and cell inflammation [10-12,22]. No study to our knowledge has examined the relationship between Cho and functional brain activity. Therefore, it is unclear whether or not inflammation, as indicated by increased Cho levels, will be associated with differences in brain activation patterns.

Methods

Participants

Data for the current study was collected as part of a larger fMRI study and has been published elsewhere (Genova et al. 2009). In the current study, data from a subset of individuals who received MRS were analyzed. Seventeen, right-handed participants (9 healthy adults (HCs) and 8 individuals with clinically definite MS (23) participated in the current study. The HCs group age ranged from 32 to 55 (M=43.1, SD=3.08) and had a mean of 15.3 years (SD=0.65) of education. The MS group age ranged from 24 to 49 (M=41, SD=2.22) and had a mean of 14.57 years of education (SD=0.57). The average time since MS diagnosis was 5.6 years (SD=1.25). Of the 8 MS subjects, 6 subjects had relapsing-remitting MS, 1 subject had chronic progressive MS, and one subject’s disease subtype was unknown at time of study. There were no significant between-group differences for age (t (15)=-0.614, p=0.549), years of education (t (15)=-0.856, p=0.407) or gender (X² (1)=0.701 p=0.402).

Prospective participants were excluded if they had a history of psychiatric illness, admission to alcohol/drug treatment program, previously diagnosed with a neurological disorder, or brain injury. MS participants were at least one-month post most recent exacerbation, if any, and were free of corticosteroid use at the time of testing.

Procedure

All procedures, including informed consent, were approved by the Institutional Review Boards of Kessler Foundation Research Center and the University of Medicine and Dentistry of New Jersey, and complied with HIPAA standards. Therefore, all procedures have been performed in accordance with the Declaration of Helsinki. All participants received monetary compensation for their participation.

Behavioral procedure

During the fMRI scan, subjects performed a modified version of the Symbol Digit Modalities Task (mSDMT; described previously [2]). Briefly, this rapid visual scanning task requires the respondent to determine if a letter/number pairing in a target matches a stimulus array provided simultaneously (Figure 1).

![Figure 1: Illustrates the modified Symbol Digit Modalities Task (mSDMT).](image-url)

Magnetic resonance imaging procedure: Neuroimaging was performed on a Siemens Allegra 3T MRI. Whole brain axial T1-weighted conventional spin-echo images (in-plane resolution=0.859 mm²) for anatomic overlays (TR/TE=450/14 ms, contiguous 5 mm,
Preprocessing of the fMRI data was performed using SPM2 software (http://www.fil.ion.ucl.ac.uk/spm2). The first nine volumes of the pre-processing steps included motion correction, realignment [24], coregistration and normalization using a 12 parameter affine approach and bilinear interpolation. Following normalization, scans were smoothed with a Gaussian kernel of 8 mm.

The data were analyzed with the Analysis of Functional NeuroImages (AFNI) software [25]. A standard motion correction procedure was performed during data preprocessing. Six motion parameters were derived: roll, pitch, yaw, and translations in the three corresponding orthogonal directions. Data points that had motion that constituted more than one (1) degree in rotation and 3.5 mm in translation were excluded from the model. Motion parameters were included in the model as regressors of no interest. Linear trends in the data were removed, and all voxels outside the brain were excluded from analysis. The raw intensity values were scaled to percent signal change. This was achieved by first computing the mean intensity value for each voxel across the entire time-series, and then (in a second step) dividing the raw intensity value at each time step by that mean, and multiplying the result by 100.

Multiple regressions were used to determine the contribution of msDMDT task performance to the observed time series data from each voxel. In order to create model time series, a standard hemodynamic response function (HRF) was convolved with a binary vector representing the timing of the onset of each msDMDT trial. Those events during which the subject responded incorrectly or failed to respond were excluded from the analysis. Because most subjects responded with 95-100% accuracy throughout the task, the number of responses excluded from the analyses was negligible.

Using the AlphaSim program (part of the AFNI suite of programs) which utilizes Monte Carlo simulations, we corrected for multiple comparisons by using an individual voxel probability threshold of p<0.01 and a minimal cluster-level threshold of 48 contiguous voxels, resulting in a corrected voxel-level probability threshold of p<0.05. In order to examine group differences in BOLD activation during performance on the msDMDT, we selected specific regions of interest (ROIs). These regions were found to be critically involved in the performance of the msDMDT in a previous investigation of processing speed [2]. Percent signal change was compared between the two groups using a t-test in the following 8 ROIs: prefrontal gyrus (including the inferior, middle, and superior frontal gyri), precentral gyrus (including supplementary motor area and medial frontal gyrus), occipital gyri (including the lingual gyrus), inferior parietal gyri (including the cuneus), cerebellum, middle temporal lobe, thalamus and cingulate gyrus. ROIs were drawn using "Draw Dataset" plugin of AFNI Suite.

Preprocessing of the 1H-MRS data required that first, the data be zero-filled from 1,024 to 2,048 points in the time domain and from 16x16 to 128x128 on the spatial domain. Each voxel was then reconstructed, frequency aligned, and phase corrected with respect to its NAA peak, as shown in Figure 2d [26]. Relative levels of the NAA, Cr and Cho in each of the 70 voxels of the VOI of each subject were estimated from their peak area, using the STTools-FITT parametric spectral modeling and least-squares optimization software of Soher et al. [27], as shown in Figure 2e.

The VOI may also contain variable amounts of CSF (Fig. 2a-c) whose metabolite concentrations are below the 1H-MRS detection threshold [28]. Ignoring the CSF fraction in the VOI, therefore, will lead to an underestimation of its concentration in the tissue of the...
VOI, which may be exacerbated in MS patients, due to their known accelerated atrophy. To correct for the VOI CSF fraction, CSFv, we produced VOI CSF masks from the axial T2-weighted true-FISP images using our in-house FireVoxel software segmentation package [29]. This software first corrects all images for nonuniform intensities due to the coil’s RF inhomogeneities, using the common histogram devolution technique of Sled et al. [30]. Each subject’s metabolites’ concentrations were subsequently divided by (1-CSFv) to correct for the CSF partial volume.

Lesion Volume

A measure of lesion burden was obtained for every subject, the methodology has been described elsewhere (Genova et al., 2009). Briefly, brains were identified using 32-slice T2 FLAIR images and verified by neuroradiologist. The lesions were manually segmented on all axial slices starting from the most superior axial slice and ending at the axial level where the posterior horn of the lateral ventricles separated from the body of the lateral ventricle. This procedure was performed in order to exclude any hyperintensities caused by air artifact at the level of the sinuses or normal white matter hyperintensities occurring in the occipital lobe. Therefore, the lesions measured were representative of "true" MS pathology and not normal variation due to artifact or individual variability present in all subjects.

Results

Behavioral results

There were no significant differences in accuracy rates on the mSDMT between the HC (M=0.97, SD=0.03) and MS group (M=0.96, SD=0.025), t (15)=0.85, p=0.41, with both groups performing at ceiling. Analysis of current reaction time (RT) data revealed that the MS group (M=2074 ms, SD=348.2) had significantly longer RT during mSDMT performance compared to the HC group (M=1588 ms, SD=249.2), t (15)=3.34, p=0.004. After controlling for potential motor slowing (by covarying out score A of the Trail-Making Test (2)), the MS group still had significantly slower reaction time than the HC group (F (1)=5.53, p=0.034).

Examination of Between-group differences in BOLD activity

Two-tailed independent-samples t-tests were performed on the ROIs in order to see whether group differences existed. Frontal and occipital ROIs showed significant group differences. Specifically, bilateral medial (right: t (15)=3.75, p<0.05, left: t (15)=2.99, p<0.01) and middle frontal gyrus (right: t (15)=3.23, left: t (15)=2.09, p=0.05) (BA6 and 9, respectively), right inferior frontal gyrus (t (15)=2.34, p<0.05), left cuneus (t (15)=2.29, p<0.01), left lingual gyrus (t (15)=2.56, p<0.05) and right superior frontal gyrus (t (15)=3.14, p=0.007) showed significantly more activity in HCs than in MS participants.

Lesion Volume

The average total lesion volume was 7.42ml in the MS subjects. Given that the VOI was 70 ml in size (of estimated 1200ml brain size), the lesions likely contributed roughly 0.5% of the VOI. Therefore, give the lesion volume of the sample, we did not correct for lesioned tissue.
appears that the fMRI activation patterns during performance of a cognitive task are related to metabolic levels.

The regions in which a positive relationship was found between NAA and BOLD activity are consistent with previous fMRI investigations of mSDMT in which occipital and frontal activity was associated with task performance (e.g. Genova et al., 2009; Forn et al., 2009, Forn et al., 2013). These findings may provide additional insight into BOLD activity patterns in individuals with MS. Our findings of a positive relationship between NAA and BOLD activity are divergent with findings related to motor task (Reddy et al., 2002) where a negative relationship was found between NAA and BOLD activity. However, there are multiple differences between our study and that of Reddy et al. (2002). For one, the task which is performed in the scanner in the current study is cognitive in nature compared to the motor task used in Reddy et al. (2002). Therefore, it may be that increased neurometabolite levels are differentially associated with BOLD activity depending on whether cognitive or motor functions are engaged. Finally, and perhaps most importantly, we did not utilize obtain neurometabolite values based on the ratio with Creatine, whereas Reddy et al. did.

Cho is thought to be an indicator of inflammation and has been reported to be elevated in individuals with relapsing-remitting MS compared to HCs [22]. In the current study, while we did not find differences between groups, we found that Cho positively correlated with BOLD activity in the occipital cortex. It is difficult to interpret why increased Cho (an indicator of inflammation) would lead to increases in BOLD activation. However, elevated Cho can also be indicative of remyelination, which may explain the increased functional activation elsewhere [12]. Due to the small sample size and the fact that no one to our knowledge has examined the relationship between choline and BOLD activation, it is difficult to make a strong conclusion regarding our findings. Regardless, our study is an important first step in the examination of this relationship.

Conclusion
The current findings contribute to a rather scarce body of literature that examines structural pathology in MS individuals in conjunction with functional brain activity (17,19,21). Since we have a very small sample size in our study and measure neurometabolite levels in only one slice, our conclusions are indeed limited. However, our findings suggest that a complex interplay of neurometabolite levels might differentially affect BOLD activation patterns. In order to investigate this further, it is critical to examine neurometabolite concentrations within specific functional brain regions. Future work should utilize larger sample sizes and whole brain MRS acquisition since it offers valuable information regarding neurometabolism (13). In addition, investigation of neurometabolite levels in relation to cognitive function in regions beyond those that were described here might clarify some of the findings we obtained. However, this research represents an important first step in examining the relationship between functional brain activity associated with cognition and neurometabolite levels.

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