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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.

Keywords Magnetic resonance spectroscopy; Functional magnetic resonance imaging; Multiple sclerosis; Processing speed

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 that signal potential inflammation [12,13]. Recently MRS has been reported to be a strong predictor of brain volume loss and disability in MS [14]. The current study utilizes both techniques (fMRI and 1H-MRS) to examine the relationship between damage to brain tissue in MS and blood-oxygen-level dependent (BOLD) activity.

Several studies have used both MRS and fMRI to examine the relationship between microstructural pathology and blood-oxygen-level dependent (BOLD) activation [15-18]. These studies have examined motor abilities in individuals with MS and have consistently shown that reductions in the neurometabolite N-acetyl-L-aspartate (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 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).

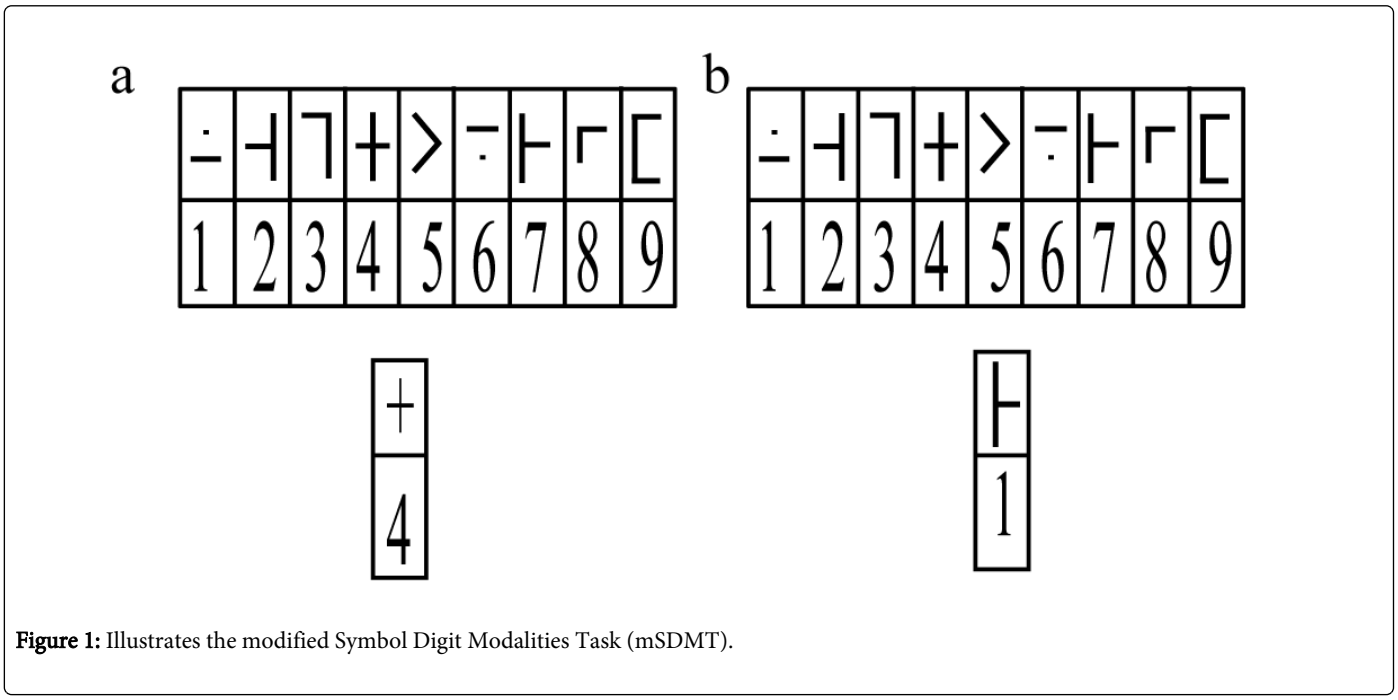

Figure 1: Illustrates the modified Symbol Digit Modalities Task (mSDMT).

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 mm^2) for anatomic overlays (TR/TE=450/14 ms, contiguous 5 mm,

256×256 matrix, FOV=24 cm, NEX=1) were obtained before acquisition of functional data. Functional imaging consisted of multislice gradient echo $T2^*$-weighted images, acquired with echoplanar imaging (EPI) methods (TE=30 ms; TR=2000 ms; FOV = 24 cm; flip angle=80°; slice thickness=5 mm contiguous, matrix= 64×64, in-plane resolution=3.75 mm^2). In order to provide coverage of the entire brain, a total of 32 contiguous slices in the axial plane were acquired.

Following the fMRI protocol, the 7 cm (left-right)10 cm (anterior-posterior)×1.5 cm (inferior-superior) MRS Volume of Interest (VOI) placement was image guided based on: (i) T1-weighted sagittal and coronal localizers: TE=16 ms, TR=500 ms, 256x128 matrix, 5 mm thick slices, no gap; (ii) a T1-weighted volume axial series (MPRAGE, TE=6.9 ms, TR=17.7 ms, flip angle=25˚, 256×192 matrix, 1.5 mm contiguous slices); and (iii) a conventional T2-weighted series (TE=100 ms, TR=2800 ms, 5 mm slices with no gap) for lesion identification. The VOI was placed one slice superior to the ventricles in order to maximize white matter fraction in VOI and minimize CSF content, as shown in Figure 2a-c. The following 1H-MRS protocol comprised the Siemens product short (TE=30ms), TR=1500 ms PRESS 2D Chemical Shift Imaging (16x16 cm^2 FOV, 16x16 phase encoding steps). At this TR the MRS acquisition took 7 minutes and the signals were acquired for 0.5 second at ± 1 KHz bandwidth.

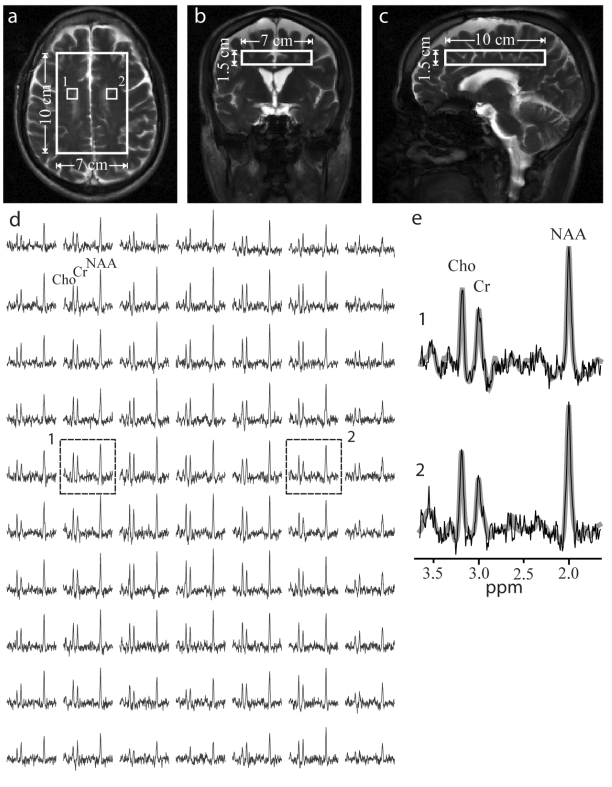

Figure 2: Illustrates MRS VOI placement.

Data analysis

Preprocessing of the fMRI data was performed using SPM2 software (http://www.fil.ion.ucl.ac.uk/spm2). The first nine volumes were removed from analyses in order to control for saturation effects. Preprocessing 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 mSDMT 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 mSDMT 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 mSDMT, we selected specific regions of interest (ROIs). These regions were found to be critically involved in the performance of the mSDMT 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 ^{1}H-MRS data required that first, the data be zero-filled from 1,024 to 2,048 points in the time domain and from 16×16 to 128×128 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 SITools-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 ^{1}H-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, CSFf, 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-CSFf) 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=348.22) 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 .5% of the VOI. Therefore, give the lesion volume of the sample, we did not correct for lesioned tissue.

Examination of Between-group differences in Neurometabolite levels

Two-tailed independent samples t-tests were performed to examine group differences in terms of neurometabolite levels of NAA and Cho. Means and standard deviations are provided in Table 1. No significant differences were found between groups in NAA or Cho.

Neurometabolite	Mean	Standard Deviation
MS		
NAA	34.87	3.25
Creatine	19.66	2.38
Choline	5.71	0.65
HC		
NAA	32.7	1.73
Creatine	17.57	0.94
Choline	17.57	0.94

Table 1: Means and standard deviations of neurometabolites in the MS and HC sample.

Examining the relationship between fMRI activation and neurometabolism

Pearson correlations were used to determine the relationship between activity in the ROIs and neurometabolite levels in the entire slice, by correlating percent signal change in these regions of interest with neurometabolite levels of concentrations.

Due to the small sample size of the study and the nonsignificant differences between groups in neurometabolite concentrations, we collapsed across groups and found positive correlation between NAA levels averaged across the entire slice and BOLD activity in the frontal and occipital ROI (bilateral inferior occipital gyrus: r=0.682, p=0.003; bilateral middle occipital: r=0.573, p=0.02; left fusiform gyrus: r=0.492, p=0.04; and right precentral gyrus (supplementary motor area; SMA): r=0.503, p=0.04).

Similarly, Cho levels positively correlated with activation in the bilateral inferior occipital: r=0.613, p=0.009, left fusiform gyrus: r=0.515, p=0.03, bilateral middle occipital gyrus: r=0.519, p=0.03.

Discussion

The main purpose of the current study was to examine the relationship between brain activity during performance of a cognitive task and neurometabolism. Our findings indicate that increased BOLD activity in frontal and occipital regions is associated with increased concentration of NAA and Cho. Specifically, this association was observed in right SMA and bilaterally in regions of the occipital cortex, even after controlling for atrophy. Although our study was a pilot study with small sample size, it has several strengths which increase our confidence in obtained results. Specifically, we corrected for MS-related pathology, in order to examine neurometabolite levels not confounded by atrophy. Additionally, we examined absolute values of neurometabolites, and not a ratio score, which is known to be affected by unstable levels of creatine in MS [22,31,32]. Taken together, it

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 activation 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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