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A R T I C L E   I N F O

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A B S T R A C T

Introduction: MS is associated with structural and functional brain alterations leading to cognitive impairments across multiple domains including attention, memory, and speed of information processing. Here, we analyzed the white matter damage and topological organization of white matter tracts in specific brain regions responsible for cognition in MS.

Methods: Brain DTI, rs-fMRI, T1, T2, and T2-FLAIR were acquired for 22 MS subjects and 22 healthy controls. Automatic brain parcellation was performed on T1-weighted images. Skull-stripped T1-weighted intensity inverted images were co-registered to the b0 image. Diffusion-weighted images were processed to perform whole brain tractography. The rs-fMRI data were processed, and the connectivity matrices were analyzed to identify significant differences in the network of nodes between the two groups using NBS analysis. In addition, diffusion entropy maps were produced from DTI data sets using in-house software.

Results: MS subjects exhibited significantly reduced mean FA and entropy in 38 and 34 regions, respectively, out of a total of 54 regions. The connectivity values in both structural and functional analyses were decreased in most regions of the default mode network and in four other cognitive networks in MS subjects compared to healthy controls. MS also induced significant reduction in the normalized hippocampus and corpus callosum volumes; the normalized hippocampus volume was significantly correlated with EDSS scores.

Conclusion: MS subjects have significant white matter damage and reduction of FA and entropy in various brain regions involved in cognitive networks. Structural and functional connectivity within the default mode network and an additional four cognitive networks exhibited significant changes compared with healthy controls.

1. Introduction

MS is an inflammatory and neurodegenerative disease of the central nervous system; more common in women than men [1]. It is the most common non-traumatic neurological disorder among young adults leading to neurological disability [2,3]. MS disrupts the integrity of the white matter [4], leading not only to physical disability but also to cognitive impairment [5-7].

A major challenge in MS studies is to understand how structural and functional networks are interrelated and how the disease may alter or damage the relationships among various regions within different cerebral networks [8]. MRI has contributed significantly to the depiction of...
anatomical and functional connectivity in MS studies. fMRI evaluates brain functions using indirect measurements of blood oxygenation level [9]. fMRI studies have demonstrated functional impairments in 30%–70% of cerebral tissue areas involving attention, memory and information processing speed in MS [10,11].

DTI is an MRI-based neuroimaging technique that characterizes microstructural tissue integrity based on diffusion properties. DTI makes it possible to estimate the location, orientation, and anisotropy of the brain’s WM tracts [12]. DTI has demonstrated WM disruptions in all forms of MS (relapsing remitting, secondary progressive, primary progressive) with a pronounced WM disruption within the corpus callosum [13]. Moreover, DTI has demonstrated in brain regions responsible for cognitive functions such as the default mode network [14,15], hippocampus, prefrontal and temporal areas [16]. Most MRI studies of MS predominantly used FA to investigate WM tract alterations in MS subjects in comparison to healthy controls [12,13]. Despite its popular application, conventional DTI has shortcomings resulting from its two underlying assumptions. First, the use of a ‘single’ diffusion tensor to characterize a pixel volume, which may contain thousands of tissue components, resulting in a diffusion tensor representing only an average of these multiple tissue compartments. Conventional DTI, thus, inappropriately yields low FA in crossing fiber regions. Second, FA is insensitive to detecting axonal density in gray matter due to the relatively random fiber orientation distribution and low axial density. To overcome these limitations of FA, we have developed the diffusion entropy approach which provides improved quantitative characterization of axonal changes especially for crossing axonal fibers [17]. We have demonstrated that entropy measurements can better characterize the axonal properties in locations containing multiple diffusion orientations, resulting in improved image contrast when compared with FA maps [17]. In the current study, we have tested the ability of diffusion entropy in detecting lesions which may involve crossing axonal bundles in MS patients.

Combining analyses of resting state connectivity and diffusion tensor imaging, researchers found improved MRI representation of brain connectivity in both healthy and pathological states [18–23]. Although sensorimotor, visual and language systems studies in MS subjects, using diffusion tensor tractography and graph theory, showed brain network topological efficiency was reduced compared to healthy controls [18], the DMN is probably the most studied network in MS. Studies of all forms of MS have found that DMN abnormalities correlate with cognitive impairment and damage to select WM fiber bundles [4,19–24].

Changes in connectivity have been hypothesized to be related to brain atrophy, deterioration in WM tracts and lesion load in MS [20–24]. In a previous study, using resting-state fMRI, we assessed brain connectivity and found abnormal connectivity links for most patients with MS in the DMN as well as in other cognitive networks including attention, memory, language, and visual–spatial memory [25].

In this study, using DTI, we have analyzed the WM tracts that directly and indirectly connect the brain regions responsible for cognition in MS subjects and compared these tracts with those of healthy controls. For this purpose, we employed FA and entropy to analyze how brain WM tracts change in MS subjects compared to healthy controls. We tested whether a combination of entropy and FA could provide additional information regarding the injured MS brain.

We also investigated brain volume changes and the correlation of both DTI and rs-fMRI connectivity with the weakening of the links in these different cognitive networks in MS subjects and compared these changes and parameters to those of healthy controls.

2. Methods

2.1. Subjects

Age-, race-, and sex-matched MS (n = 22) and healthy controls (n = 22) participated in this study. The inclusion criteria included subjects with a MS diagnosis according to the revised 2010 McDonald criteria and a relapsing-remitting or secondary progressive form with active disease. The exclusion criteria were as follows: 1) relapse occurring within 1 month before MRI scan; 2) steroid treatment within 1 month prior to enrollment or changes in disease modifying treatment within 6 months before MRI scan; 3) primary-progressive MS; 4) subjects with artifacts or noise in the raw MRI data.

Brain MRIs were obtained for all MS subjects and healthy controls. All MS subjects underwent a full neurological examination within one month prior to MRI and their EDSS scores were recorded by a MS specialist. Electronic medical records were reviewed, and data were collected on diagnosis criteria, socio-demographic factors, neurological and cognitive symptoms. The study protocol was approved by the Henry Ford Health System Institutional Review Board; written informed consent was obtained from all subjects.

2.2. MRI acquisition

MRI was performed on a GE 3.0-T whole-body magnet using an eight channel phase array head coil. rs-fMRI images were acquired using a gradient echo EPI sequence with a FOV of 22 × 22 cm on a 64 × 64 matrix, 34 slices/3.5 mm slice thickness, TR/TE = 2000 ms/300 ms. One hundred fifty volumes (five minutes) were recorded while the participants were lying quietly with eyes open inside the scanner and using ear plugs for minimizing the noise from the scanner. Keeping the eyes open eliminates the coherent activity in the occipital cortex and leads to more reliable results compared to the situation where the eyes are closed [26,27]. Respiration and cardiac data of the participant were recorded during the scan using a pneumotatic belt and pulse-oximeter, respectively. Artifact removal from the fMRI images was performed using these data, prior to image analysis.

To segment the brain images, high resolution inversion pulse prepared spoiled GRASS (IRSPGR) three-dimensional images were acquired with FOV of 24 × 24 cm, pixel dimensions 0.94 × 0.94 × 1 mm, 142 slices/1 mm slice thickness, TR/TE = 8.816 ms/3.496 ms. To detect the MS lesions, T2-FLAIR images were acquired with FOV of 24 × 24 cm, TR/TE = 8500 ms/80 ms, TI = 2550ms, imaging matrix 256 × 256 with pixel dimensions 0.94 × 0.94 mm², 32 slices/4 mm slice thickness.

Also q-ball images were acquired with 55 diffusion gradient directions with b-value = 1500/s/mm², performed using spin echo diffusion-weighted 2D echo-planar imaging with FOV = 24 cm, TR/TE = 8 s/94 ms, slice thickness of 2.6 mm, and in-plane pixel 2.5 × 2.5 mm² and 96 × 96 imaging matrix interpolated to 0.975 × 0.975 mm² and 256 × 256 matrix.

2.3. Image analysis

2.3.1. Functional networks

In our previous study [25], we performed a whole brain functional connectivity analysis and investigated five circuits in the brain responsible for cognitive functions in MS subjects, as follows:

- **Default Mode Network (DMN):** Medial prefrontal cortex, rostral anterior cingulate cortex, posterior cingulate cortex, precuneus and lateral parietal cortex.
- **Attention:** Thalamus (superior colliculus and pulvinar), superior parietal lobule, middle frontal gyrus, superior frontal gyrus, angular gyrus and supramarginal gyrus.
- **Verbal memory:** Inferior temporal gyrus, medial occipitotemporal gyrus, middle temporal gyrus, temporal pole, angular gyrus, inferior frontal gyrus.
- **Memory:** Angular gyrus, fusiform gyrus, superior parietal lobule, supramarginal gyrus, hippocampal formation, and superior frontal gyrus.
• **Visual-Spatial Working Memory:** Cingulate region, superior frontal gyrus, fornix, superior parietal lobule, and supramarginal gyrus.

Here, using DTI, we analyzed the WM tracts of brain regions which are responsible for cognition and compared them between MS subjects and healthy controls to determine whether MS could elicit a meaningful change. Furthermore, we investigated the correlation between the rs-fMRI connectivity values and the weakening of links in these networks. A summary diagram demonstrating the steps performed in this study to calculate the connectivity matrix for each subject is shown in Fig. 1.

2.3.2. Brain parcellation

Automatic brain parcellation was done on T1-weighted images employing the FreeSurfer package [28]. For each case, T1-weighted images were processed into several cortical and subcortical regions and labeled. Among them, 82 regions per hemisphere were extracted including 74 cortical and 8 subcortical nodes in each hemisphere. The subcortical regions were the thalamus, caudate nucleus, putamen, globus pallidus, hippocampus, amygdala, nucleus accumbens, and ventral diencephalon. The resulting maps were inspected visually for any notable errors that might have occurred during skull stripping or brain segmentation and parcellation steps.

2.3.3. Co-registration

Skull-stripped T1-weighted intensity inverted images were coregistered to the b0 image using coarse registration in FSL [29]. The resulting transformation was applied to the parcellation label map to transform the labels to the DTI space. A similar procedure was performed to transform the labels to the fMRI space. The performance of the co-registration step was inspected and validated visually.

2.3.4. DTI connectivity

Diffusion-weighted images were processed using MRtrix tool [30] to perform whole brain tractography. First, a mask was generated from DTI to extract the brain. Then, diffusion tensors were estimated, and FA maps were calculated. Voxels with a high FA value (i.e., higher than 0.7) were considered to belong to single fibers and used to build the diffusion-weighted signal response profile of a single fiber. By deconvolving this response from DTI data using spherical harmonics of order 6, a DTI orientation map was calculated. Using the FA and orientation maps and starting from a dilated WM mask, the program performed a whole brain probabilistic tractography to generate 150,000 fibers longer than 10 mm and avoided entering voxels with an FA value less than 0.1. Having the whole fibers and the co-registered label image, the connectivity matrix was built for each participant by calculating the connectivity strength between each pair of regions (i,j, i,j \in 1,...,164). The connectivity was calculated by averaging the mean-FA value of all the fibers passing through or connecting the two regions. The mean-FA value of each fiber was also derived by averaging the FA values of the voxels it passed through.

2.3.5. rs-fMRI connectivity

Pre-processing of the rs-fMRI data was done using FSL which includes eliminating the first five volumes due to magnetization equilibrium, brain extraction, motion correction, slicing timing, temporal high-pass filtering, and spatial smoothing (FWHM = 5 mm). To calculate the functional connectivity matrix for each participant, we used the same anatomical label map resulting from FreeSurfer that was co-registered to the fMRI space. The rs-fMRI connectivity of each pair of ROIs was measured and yielded the 164 × 164 rs-fMRI connectivity matrix.

2.3.6. GroupWise comparison

Using NBS [31] the connectivity matrices of healthy controls and MS patients were analyzed statistically to find any significant differentiating network of nodes between the two groups.

2.4. Statistical analysis

For continuous variables, the mean and standard error (SE) are reported. For categorical variables, the number and percentage are reported. We used two-sample, two-tailed t-tests and Fisher exact tests, as appropriate, to statistically compare MS subjects to healthy controls. Pearson’s correlation coefficients were employed to statistically correlate EDSS and brain volumes. Statistical significance was established at a
p-value less than the critical alpha value of 0.05 (2-tailed). However, we used adjusted critical alpha values, calculated using the conservative Bonferroni method, when making multiple statistical comparisons. Normalized brain region volume is calculated as a percentage of whole brain volume. All statistical analyses were conducted using STATA 14.2 for Mac (College Station, TX). The rs-fMRI connectivity of each pair of ROIs was measured by calculating the Pearson correlation of their averaged time series and re-scaled using the Fisher z-transform.

3. Results

3.1. Study population /Subjects

Table 1 shows the demographic characteristics of the study participants. At baseline, the mean (SE) age for 22 MS subjects was 38.55 (2.16) years; 68.2 % were white and 77.3 % were female. The mean (SE) disease duration for these MS subjects was 9.82 (7.87) years and the mean (SE) EDSS score was 2.86 (2.35). The percentage of these MS subjects with cognitive complaints was 22.7 % and the percentage on disease-modifying therapy was 81.8 %. The mean (SE) age for 22 subjects with cognitive complaints was 22.7 % and the percentage on disease-modifying therapy was 81.8 %.

Normalized brain region volume

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>MS(n = 22)</th>
<th>Control(n = 22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean(SE)</td>
<td>38.55(2.16)</td>
<td>39.24(2.50)</td>
<td>0.8</td>
</tr>
<tr>
<td>Race, n(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>15(68.2 %)</td>
<td>8(47.1 %)</td>
<td>0.2</td>
</tr>
<tr>
<td>Other</td>
<td>7(31.8 %)</td>
<td>9(52.9 %)</td>
<td></td>
</tr>
<tr>
<td>Gender, n(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>17(77.3 %)</td>
<td>16(72.7 %)</td>
<td>0.7</td>
</tr>
<tr>
<td>Male</td>
<td>5(22.7 %)</td>
<td>6(60.0 %)</td>
<td></td>
</tr>
</tbody>
</table>

MRI volume measurements

<table>
<thead>
<tr>
<th>Whole brain volume in mm^3, mean(SE)</th>
<th>MS(n = 22)</th>
<th>Control(n = 22)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thalamus</td>
<td>1,159,091</td>
<td>1,172,727</td>
<td>0.7</td>
</tr>
<tr>
<td>White</td>
<td>32,003</td>
<td>17,632</td>
<td></td>
</tr>
<tr>
<td>Normalized thalamic volume in %, mean(SE)</td>
<td>0.80(0.04)</td>
<td>0.77(0.02)</td>
<td>0.6</td>
</tr>
<tr>
<td>Normalized white matter volume in %, mean(SE)</td>
<td>0.40(0.01)</td>
<td>0.40(0.004)</td>
<td>0.7</td>
</tr>
<tr>
<td>Normalized CSF volume in %, mean(SE)</td>
<td>20.29(0.20)</td>
<td>20.26(0.18)</td>
<td>0.9</td>
</tr>
<tr>
<td>Normalized hippocampus volume in %, mean(SE)</td>
<td>0.37(0.01)</td>
<td>0.40(0.01)</td>
<td>0.02</td>
</tr>
<tr>
<td>Normalized corpus callosum volume in %, mean(SE)</td>
<td>0.62(0.02)</td>
<td>0.70(0.02)</td>
<td>0.003</td>
</tr>
<tr>
<td>Normalized lateral ventricle volume in %, mean(SE)</td>
<td>1.22(0.05)</td>
<td>1.17(0.04)</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Clinical features

| Disease duration in years, mean(SE) | MS(n = 22) | 9.82(7.87) |         |
| EDSS score, mean(SE)               | 2.86(2.35) |           |         |
| Cognitive complaints(Y/N), n(%)    | 5(22.7 %)  |           |         |
| Use of disease-modifying therapies (Y/N), n(%) | 19(81.8 %) |           |         |

*Indicates p-value significant at a critical alpha level of 0.05.

3.2. MRI findings and clinical characterisation

3.2.1. Brain region volume alteration

The mean (SE) whole brain volume did not differ between MS subjects and healthy controls (0.40 % (0.01 %) and 0.70 % (0.02 %), respectively) as compared to healthy controls (0.40 % (0.01 %) and 0.70 % (0.02 %), respectively). MS subjects and healthy controls did not differ in terms of normalized thalamic (p = 0.6), WM (p = 0.7), CSF (p = 0.9), and lateral ventricle (0.4) volumes (Table 1).

3.2.2. FA and entropy findings

3.2.2.1. Default mode network. The FA value in the DMN decreased significantly in the right/left cingulate region (p < 0.0001), right/left pericuneus (p < 0.0001/p = 0.01), and right/left parietal lobe (p = 0.0001/p = 0.0002) in MS subjects compared to healthy controls (Fig. 2A). Entropy also decreased significantly in the right/left cingulate region lobe (p = 0.0003/p = 0.0001), right/left pericuneus lobe (p = 0.0008/p = 0.02), in MS subjects compared to healthy controls (Fig. 3A).

3.2.2.2. Attention. The FA value in the attention network decreased significantly in the right/left thalamus (p = 0.004/p = 0.05), right/left angular gyrus (p < 0.0001/p = 0.0001), right/left supramarginal gyrus (p < 0.0001) and increased significantly in the right/left superior parietal lobule (p < 0.0001) in MS subjects compared to healthy controls (Fig. 2B). Entropy also decreased significantly in the right/left thalamus (p = 0.001/ p = 0.003), right angular gyrus (p = 0.0001), right/left supramarginal gyrus (p < 0.0001) and increased significantly in the right/left superior frontal gyrus (p = 0.0001) and superior parietal lobule (p < 0.0001) in MS subjects compared to healthy controls (Fig. 3B).

3.2.2.3. Verbal memory. The FA value in the verbal memory network decreased significantly in the right/left angular gyrus (p < 0.0001/p = 0.0001), right/left inferior temporal gyrus (p < 0.0001), right/left medial occipitotemporal gyrus (p < 0.0001), right/left middle temporal gyrus (p < 0.0001), right/left temporal pole (p < 0.0001), right/left inferior frontal gyrus (p < 0.0001) in MS subjects compared to healthy controls (Fig. 2C). Entropy also decreased significantly in the right angular gyrus (p < 0.0001), right/left inferior temporal gyrus (p < 0.0001), right/left medial occipitotemporal gyrus (p = 0.0008/p = 0.003), right/left middle temporal gyrus (p < 0.0001), right/left temporal pole (p < 0.0001), right/left inferior frontal gyrus (p < 0.0001) in MS subjects compared to healthy controls (Fig. 3C).

3.2.2.4. Memory. The FA value in the memory network decreased significantly in the right/left fusiform gyrus (p < 0.0001), right/left angular gyrus (p < 0.0001/p = 0.0001), right/left supramarginal gyrus (p < 0.0001), right/left hippocampal formation pole (p < 0.0001) and increased significantly in both the right and left superior parietal lobule (p < 0.0001) in MS subjects compared to healthy controls (Fig. 2D). Entropy also decreased significantly in the right angular gyrus (p < 0.0001), right/left supramarginal gyrus (p < 0.0001), right/left hippocampal formation pole (p = 0.0002/p = 0.0001), right/left fusiform frontal gyrus (p < 0.0001) and increased significantly in the right superior parietal lobule (p < 0.0001), right/left superior frontal gyrus (p < 0.0001) in MS subjects compared to healthy controls (Fig. 3D).

3.2.2.5. Visual-spatial working memory. The FA value in the visual-spatial working memory network decreased significantly in the right/left cingulate region (p < 0.0001), right/left supramarginal gyrus (p < 0.0001) and right/left fornix (p = 0.0001/p = 0.0001) and increased significantly in the superior parietal lobule (p < 0.0001) in MS subjects compared to healthy controls (Fig. 2E). Entropy decreased significantly in the right/left cingulate region (p = 0.0003/p = 0.0001), right/left supramarginal gyrus (p < 0.0001), right/left fornix (p = 0.0004/p = 0.0005) and increased significantly in the right/left superior parietal lobule (p < 0.0001) and right/left superior frontal gyrus (p < 0.0001) in MS subjects compared to healthy controls (Fig. 3E).
3.2.3. Structural and functional connectivity alteration

Fig. 4 shows the disrupted structural and functional components in MS subjects. The results for each network is described below.

3.2.4. Structural connectivity alteration

3.2.4.1. Default mode network. The DTI connectivity in the DMN decreased between the anterior cingulate cortex with the lateral parietal cortex in the right hemisphere and between the left posterior cingulate cortex with the prefrontal cortex, anterior cingulate cortex and lateral parietal cortex in the right hemisphere, but increased between the left anterior cingulate cortex with the lateral parietal cortex and anterior cingulate cortex in the right hemisphere in MS subjects compared to healthy controls (Fig. 4A).

3.2.4.2. Attention. The DTI connectivity in the attention network decreased between the frontal gyrus with the thalamus (superior colliculus and pulvinar) in the left hemisphere and between the right frontal gyrus with the left occipitotemporal gyrus and inside the temporal gyrus but it increased between the temporal gyrus with both the temporal pole and frontal gyrus on the left side and the angular gyrus on the right side in MS subjects compared to healthy controls (Fig. 4B).

3.2.4.3. Verbal memory. The DTI connectivity in the verbal memory network decreased between the temporal gyrus with the temporal pole, the occipitotemporal gyrus with the angular gyrus and the temporal pole with the frontal gyrus in the left hemisphere. It also decreased between the right occipitotemporal gyrus with the angular gyrus and frontal gyrus on the left side, between the right frontal gyrus with the left occipitotemporal gyrus and inside the temporal gyrus but it increased between the temporal gyrus with both the temporal pole and frontal gyrus on the left side and the angular gyrus on the right side in MS subjects compared to healthy controls (Fig. 4C).

3.2.4.4. Memory. The DTI connectivity in the memory network decreased between the frontal gyrus with the angular gyrus and supramarginal gyrus in the left hemisphere and between the right fusiform gyrus with the left frontal gyrus. It also decreased between the right supramarginal gyrus with the angular gyrus, hippocampal formation and frontal gyrus in the left hemisphere and between the right frontal

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Fig. 2. Mean (SE) FA for different networks in MS subjects vs. healthy controls. A) DMN; B) Attention; C) Verbal Memory; D) Memory; E) Visual-Spatial Memory in MS subjects vs. healthy controls.
gyrus with the left parietal lobule, left supramarginal gyrus, right angular gyrus and right fusiform gyrus but increased between the right parietal lobule with the fusiform gyrus and superior frontal gyrus on left side and between the right frontal gyrus with the left hippocampal formation and right supramarginal gyrus in MS subjects compared to healthy controls (Fig. 4D).

3.2.4.5. Visual–Spatial working memory. The DTI connectivity in the visual–spatial working memory network decreased between the supramarginal gyrus with the parietal lobule in the left hemisphere, between the right frontal gyrus with both the parietal lobule and supramarginal gyrus in the left hemisphere and inside the cingulate region. It also decreased between the supramarginal gyrus with the frontal gyrus in the right hemisphere and between the fornix with the cingulate region, frontal gyrus and parietal lobule on the left side but increased between the left frontal gyrus with the parietal lobule and supramarginal gyrus in the right hemisphere and also between the cingulate region with the supramarginal gyrus on the right side in MS subjects compared to healthy controls (Fig. 4E).

3.2.5. Functional connectivity alteration

3.2.5.1. Default mode network. The fMRI connectivity in the DMN decreased between the anterior cingulate cortex with the prefrontal cortex, posterior cingulate cortex and lateral parietal cortex in the left hemisphere but increased between the precuneus with the posterior cingulate cortex and lateral parietal cortex in the left hemisphere and also between the left posterior cingulate cortex with the right posterior cingulate cortex in MS subjects compared to healthy controls (Fig. 4A).

3.2.5.2. Attention. The fMRI connectivity in the attention network decreased between the thalamus (superior colliculus and pulvinar) with the frontal gyrus and supramarginal gyrus on the left side but increased between the frontal gyrus with the parietal lobule, angular gyrus and supramarginal gyrus on the left side and also between the left frontal gyrus with the right thalamus (superior colliculus and pulvinar) in MS subjects compared to healthy controls (Fig. 4B).

3.2.5.3. Verbal memory. The fMRI connectivity in the verbal memory network decreased between the occipitotemporal gyrus with the temporal gyrus, temporal pole and angular gyrus on the left side and with the occipitotemporal gyrus on the right side. It also decreased between

Fig. 3. Mean (SE) Entropy for different networks in MS subjects vs. healthy controls. A) DMN; B) Attention; C) Verbal Memory; D) Memory; E) Visual-Spatial Memory in MS subjects vs. healthy controls.
Moreover, there is an increase between the fornix with the parietal lobule and supramarginal gyrus on the left side in MS subjects compared to healthy controls (Fig. 4E).

3.2.6. Clinical correlations

The normalized lateral ventricle volume was positively correlated with EDSS, \( r = 0.47, p = 0.03 \) (Fig. 5A). The normalized hippocampus volume was negatively correlated with EDSS, \( r = -0.49, p = 0.02 \) (Fig. 5B). There was no statistically significant correlation between EDSS and each of: whole brain volume (\( p = 0.8 \)), normalized thalamic volume (\( p = 0.08 \)), normalized WM volume (\( p = 0.4 \)), normalized CSF volume (\( p = 1.0 \)), and normalized corpus callosum volume (\( p = 0.3 \)) in MS subjects (Table 2).

4. Discussion

In this study, we employed MRI measurements of FA, entropy and rs-fMRI to investigate the structural and functional brain changes in MS. Our data suggests that MS induced significant WM damage, measured by FA and entropy in brain regions involved in cognitive networks. Although entropy improves the Gaussian model error in FA, it did not show an added advantage in detecting MS-induced lesions in our current study. Detailed investigations of structural and functional connectivity in five cognitive networks showed significant differences between MS subjects and healthy controls.

Consistent with previous investigations [32–38], we found that FA was decreased in 38 (out of 54) regions of the five cognitive networks, and a clear trend towards a decrease in 8 regions in MS subjects compared with healthy controls. However, we also found that the FA values increased in some parts of both the right and left superior parietal lobule. FA increase in MS subjects has been reported [39–46]. Although the interpretation of an increase in DTI diffusivity parameters in Gaussian models is still a topic of debate, the reasons for this discrepancy may be related to the choice of ROIs, the improvement in processing steps, and the heterogeneity of the sample size [47]. In addition, FA from Gaussian models of DTI may encounter calculation errors in the areas with fiber crossing, which can affect FA measurements. Moreover, MS is likely to reduce the thickness of myelin sheaths; Therefore, the volume of the extracellular compartment was reduced as well as increased transmembrane diffusion due to the thinner myelin sheaths, which can cause an increase in axon coherence. Increased axon coherence leads to increased FA by facilitating diffusion along the axons. These simplified scenarios do not consider the interactions between these tissue characteristics and neglect other tissue components such as glial cells and blood vessels [48]. Another reason could be a possible reactive structural and neuroplastic/responsive re-organization of the other fiber tracts [49,50] in order to compensate for the role of regional paths, whose connectivity were structurally or functionally altered in MS.

Mean entropy values decreased significantly in 34 (out of 54) regions and a trend towards a decrease in 10 regions in MS subjects compared to healthy controls. Entropy increased in some other regions including the superior frontal gyrus and the superior parietal lobule. This increase may have similar reasons as those mentioned above for FA increase. Moreover, the diffusion entropy calculation is very sensitive to noise. Our results did not demonstrate an added advantage of entropy over FA in detecting MS induced axonal changes. FA is a traditional MRI measure of axonal changes. However, due to the assumption of the Gaussian diffusion tensor model, an overall artificial lowering of FA occurs when WM fiber tracts cross [51]. Q-space diffusion tensor imaging (q-DTI), such as entropy, can better detect axonal changes, especially during the early stage which involve less organized, randomly oriented axons [19,52,53]. The reasons for the relatively reduced sensitivity of entropy in detecting axonal lesions after MS compared to FA may be attributed to the underestimated FA values of axonal reduction in the areas with crossing axonal bundles. The regions with less reduction entropy values compared to FA values may involve early stage damage with crossing axonal bundles. Whether the differences in
Our study has provided some new information. Compared to others, detection sensitivity between FA and entropy is related to the stage of the MS-induced lesions needs to be further investigated.

We also investigated the structural and functional connectivity alterations in all of the above-mentioned cognitive networks using DTI and fMRI modalities. Structural and functional connectivity in MS subjects decreased in most regions of the five cognitive networks. DTI connectivity decreased in all regions of the investigated five cognitive networks while fMRI connectivity also decreased in most regions of five cognitive network. However, fMRI connectivity increased in some regions including the precuneus, which showed much more mixed results compared to the structural connectivity. The results for the DMN are comparable with other recent studies. Our results are also comparable with other recent studies. For the other networks, Louapre et al. showed functional connectivity increased in cognitive preserve patients versus controls in the attentional networks. Castellazzi, et al. showed higher functional connectivity in the sensory motor networks area MI (SMNm1) and the medial visual network (MVN) in MS subjects compared to healthy controls. Sharbella et al. showed functional connectivity decreased in several network regions including the cerebellar, executive–control, medial–visual, basal ganglia and sensorimotor regions. Microstructural damage correlated with functional connectivity in the cerebellar and auditory networks in RR–MS compared to healthy controls has been reported by them.

We also investigated the brain volumes and compared the results between MS subjects and healthy controls. The normalized hippocampus and corpus callosum volumes were significantly reduced in MS subjects compared to healthy controls. In our current study, we did not find thalamus volume changes, which is a strong predictor of cognitive performance in MS; However, we found that normalized hippocampus volume is correlated negatively with patient’s EDSS (smaller hippocampus, higher EDSS/disability), which can be counted as a measure of brain atrophy. Higher EDSS was associated with physical disability and cognitive impairment in MS. Moreover, the hippocampus is the key region known for cognitive functional impairments and according to a study by Kohler et al., the left hippocampus volume is a strong predictor for verbal memory impairment in MS. Microstructural degeneration of the fronto-striatal-thalamic loops as well as multiple WM tract abnormalities have been postulated to be the underlying cause for cognitive impairments in MS.

In our MS cohort, only 20% of patients reported cognitive impairment. Our analyses of five cognitive networks may be more sensitive to analyze MS changes in cognitive networks compared to patient-perceived clinical symptoms. This adds evidence to a previous study which demonstrated hippocampal-thalamic-prefrontal disruption in MS patients with minimal cognitive impairment, which had postulated that this disruption may be an early manifestation of verbal memory alterations in patients who are not yet clinically impaired.

Our study has provided some new information. Compared to others, we have investigated four additional cognitive networks other than DMN, and found both structural and functional connectivity alterations in MS subjects compared to healthy controls. We also employed both FA connectivity reduction in both the middle and bilateral hippocampus and left amygdala in MS subjects compared to healthy controls. Lower FA in pediatric–onset MS group compared to healthy controls within the entire WM skeleton, particularly the corpus callosum, posterior thalamic radiation, corona radiate and sagittal stratum, and higher functional connectivity involving the anterior cingulate cortex, right precuneus of the DMN, as well as the anterior cingulate cortex and left middle frontal gyrus of the front parietal network have been reported by Akbar et al. [63].

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and entropy values to generate additional information about the stage of WM organization in the injured brain. Moreover, we investigated the evolution of structural and functional changes as well as volume changes in different regions of the brain between MS subjects and healthy controls. These results are very useful to understand how structural and functional networks are interrelated, and how MS may alter or damage the relationships among the various regions within different cerebral networks in clinical trials.

Our study has some limitations. Although the number of the MS subjects in our study can provide statistical results, it is a bit lower than some of the similar studies in this field [11–21,69–75]. The relatively small sample size may be one of the reasons for the insignificant findings related to the brain and thalamus volumes between MS subjects and healthy controls. Another limitation is the lack of clinical cognitive testing in our MS cohort to correlate with the MRI findings. Further studies are warranted using follow-up visits. Moreover, evaluating causal connectivity will be valuable.

5. Conclusion

MS subjects exhibit significant WM damage and reduction in various regions in the brain involved in cognitive networks. Normalized hippocampus volume and corpus callosum volume were significantly reduced in MS subjects compared to healthy controls.

We reported significant differences in both structural and functional connectivity within the cognitive networks of MS subjects compared to healthy controls. Analyses of both functional and structural connectivity of the five cognitive networks (DMN, attention, verbal memory, memory, and visual–spatial memory) showed significantly decreased structural connectivity bilaterally in MS subjects along with decreased functional connectivity of the superior marginal gyrus, angular gyrus, fusiform gyrus, temporal pole, hippocampal formation and thalamus. Functional connectivity decreased in both white and gray matters, while structural connectivity most likely related to WM damage in MS subjects compared to healthy controls. Some regions in the studied networks had heightened connectivity, which may represent compensatory connections as postulated in the literature [63–66].

Author’s contribution

A.L. and E.D. contributed equally and share the first authorship. Q.J. and M.C. share the senior authorship.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest in this research.

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