Increased connectivity of hub networks and cognitive impairment in multiple sclerosis

ABSTRACT

Objective: To investigate default-mode network (DMN) and frontoparietal network (FPN) dysfunction in cognitively impaired (CI) patients with multiple sclerosis (MS) because these networks strongly relate to cognition and contain most of the hubs of the brain.

Methods: Resting-state fMRI and neuropsychological assessments were performed in 322 patients with MS and 96 healthy controls (HCs). Patients with MS were classified as CI (z score < −2.0 on at least 2 tests; n = 87), mildly cognitively impaired (z score < −1.5 on at least 2 tests and not CI; n = 65), and cognitively preserved (CP; n = 180). Within-network connectivity, connectivity with the rest of the brain, and between-network connectivity were calculated and compared between groups. Connectivity values were normalized for individual means and SDs.

Results: Only in CI, both the DMN and FPN showed increased connectivity with the rest of the brain compared to HCs and CP, with no change in within- or between-network connectivity. Regionally, this increased connectivity was driven by the inferior parietal, posterior cingulate, and angular gyri. Increased connectivity with the rest of the brain correlated with worse cognitive performance, namely attention for the FPN as well as information processing speed and working memory for both networks.

Conclusions: In CI patients with MS, the DMN and FPN showed increased connectivity with the rest of the brain, while normal within- and between-network connectivity levels were maintained. These findings indicate that cognitive impairment in MS features disturbed communication of hub-rich networks, but only with the more peripheral (i.e., nonhub) regions of the brain.

Translate text to a natural representation.
Although a few studies have separately investigated such connectivity changes between resting-state networks (RSNs) in MS, no study has investigated these 3 connectivity measures together in relation to cognitive dysfunction.

The aim of the present study was therefore to investigate changes in the DMN and FPN in patients with MS with different severities of cognitive impairment by specifically investigating within- and between-network connectivity as well as connectivity with the rest of the brain.

METHODS Participants. All participants were part of the Amsterdam MS cohort, which consists of 352 patients with clinically definite MS (age 48.1 ± 11.0 years, disease duration 14.6 years, range 4.6–45.9 years) and 96 matched healthy controls (HCs; age 45.9 ± 10.5 years). Of the patients with MS, 243 patients were diagnosed with relapsing-remitting MS, 53 patients with secondary progressive MS, and 36 with primary progressive MS.

Standard protocol approvals, registrations, and patient consents. The study protocol was approved by the local ethics review board, and all participants gave written informed consent before participation.

Neuropsychological assessment and cognitive groups. On the day of scanning, all participants underwent a neuropsychological assessment as previously described, consisting of an expanded Brief Repeatable Battery of Neuropsychological tests (see e-Methods at Neurology.org). Cognitive scores were regression-adjusted for normal effects of age, sex, and educational level on the basis of the effects observed in our HCs. These corrected cognitive test scores were used to compute z scores relative to the mean and SD of HCs. Patients were defined cognitively impaired (CI) if they scored at least 2 SDs (i.e., z score < −2.0) below HCs on at least 2 cognitive tests. Patients who did not fulfill the criteria for CI but who scored at least 1.5 SDs below HCs on at least 2 domains were defined mildly CI (MCI).

Structural brain measures. White matter (WM) lesions were segmented on FLAIR images with the use of a previously described automated segmentation technique and used to calculate lesion volumes. The quality of the segmentation was manually assessed. Lesion filling (LEAP; Lesion Automated Preprocessing) was applied on the 3-dimensional T1 to minimize the effect of WM lesions on volumetric measurements. Total gray matter (GM) volume (GMV), total WM volume (WMV), and whole brain volume were then quantified with the lesion-filled T1-weighted image with SIENAX (part of FSL, www.fmrib.ox.ac.uk). In addition, deep GMV (using FIRST, also part of FSL) and cortical GMV (by removing FIRST regions from the GM mask) were computed. All volumes were normalized for head size.

fMRI preprocessing. Preprocessing consisted of removal of nonbrain tissue, motion correction, spatial smoothing with a 5-mm full width at half maximum gaussian kernel, and high-pass temporal filtering equivalent to 0.01 Hz. All resting-state fMRI scans were checked for registration errors and artifacts. The average motion did not exceed 3 mm (i.e., one voxel) for any participant; the average was 0.08 mm (0.05 mm) with no difference between HCs and MS (p = 0.34). Registration parameters were calculated with the use of boundary-based registration between the fMRI and 3-dimensional T1 sequences and non-linear registration between 3-dimensional T1 and standard space for subsequent atlas-based steps.

Regions of interest. The brain was separated into regions of interest (ROIs) with a custom-made native-space atlas as reported previously. The cortical atlas was derived from the standard-space Automated Anatomical Labeling atlas, which was coregistered to the participant’s T1-weighted scan with inverted nonlinear registration parameters and nearest-neighbor interpolation. After registration, this cortical atlas was masked by a GM mask derived from SIENAX to ensure that only cortical GM was included. Deep GM structures (i.e., thalamus, caudate, putamen, pallidum, hippocampus, amygdala, and nucleus accumbens) were segmented with FIRST on the participant’s T1-weighted scans and added to the cortical atlas. The complete atlas was subsequently coregistered to the participant’s resting-state fMRI scan with an inverted boundary-based registration matrix and nearest-neighbor interpolation to optimize registration. However, approaches such as bias-field measurements could further improve registration. After registration to IMRI, only those ROIs with at least 30% voxels remaining after registration and masking with a custom-made IMRI mask were included in the analyses. This custom-made mask was created to remove any residual nonbrain tissue and to reduce the effect of echo planar imaging distortions by excluding voxels with signal intensities in the lowest quartile of the robust range. On the basis of these criteria, 12 ROIs were excluded, made up of orbitofrontal areas and nucleus accumbens. The final atlas therefore segmented the IMRI sequence into 80 regions for which mean time series were obtained.

To determine which atlas regions make up the DMN and FPNI, the resting-state data were transferred to standard space to be able to run an independent component analysis with MELODIC. The concatenated IMRI dataset was decomposed into 42 components, with a clearly identifiable DMN and FPNI. The following regions were selected for the DMN: medial superior frontal gyrus, posterior cingulate, angular gyrus, and precuneus. For the FPNI, middle frontal gyrus, superior frontal gyrus, inferior parietal gyrus, angular gyrus, and precuneus were selected. Regions that were not part of the
DMN or FPN were considered to be the rest of the brain, consisting mainly of nonhub regions.

**Connectivity scores.** For each participant, Pearson correlation coefficients were calculated between all 80 regions to construct connectivity matrices. Given the controversial nature of negative correlations, 2 approaches were used: either set to zero or maintained in the matrices. Each connectivity score was normalized on the basis of the mean and SD of each participant’s connectivity matrix. In effect, these relative connectivity scores reflect whether connections were stronger (positive z score) or weaker (negative z score) than the average strength of all connections (z score = 0) within an individual functional network. This measure was chosen to specifically look at the ranking of each brain region within its individual brain network, in doing so correcting for gross interparticipant variability in mean connectivity. Three different measures were calculated for the DMN and FPN: the connectivity within these RSNs, the connectivity of these RSNs with the rest of the brain, and the connectivity between these RSNs (figure 1). Subsequently, a post hoc regional analysis was performed for each significant network measure to identify which brain region within the DMN or FPN was most responsible statistically for the measured connectivity changes.

**Statistical analyses.** All statistical analyses in this study were performed with SPSS 21.0 (Chicago, IL). Variables were checked for normality with Kolmogorov-Smirnov testing and histogram inspection. Data were investigated with χ² tests for categorical variables, Kruskal-Wallis tests for nonnormally distributed variables (Mann-Whitney U tests as post hoc test), or general linear models for normally distributed variables. The Levene test for equality in variances was used to assess homoscedasticity. If this assumption was violated, the Welch test was used. To investigate whether the observed connectivity changes were specific for cognitive function, the analysis was repeated with the use of groups based on Expanded Disease Severity Scale (EDSS) scores <3, 3 to 4, and >4 (i.e., based on tertiles). All general linear models were corrected for age, sex, and education. To limit the number of statistical tests, group comparisons were limited to cognitive MS groups vs controls and CI vs CP. fMRI measures that were different between groups were correlated with structural measures and clinical data with the use of Pearson correlations for normally distributed variables or Spearman correlations otherwise. Only Bonferroni-corrected p values were reported, and values of p < 0.05 were considered significant.

**RESULTS** Demographical, clinical, and MRI data. Of all patients, 180 (54%) were defined as CP, 65 as MCI (20%), and 87 as CI (26%) (table 1). As expected, CI patients were older relative to HCs and CP patients and had a longer symptom duration, higher EDSS, and lower level of education relative to CP patients. Additionally, MCI and CI patients had a lower level of education compared to HCs. There were more women in the CP group compared to HCs and CI patients. Among the volumetric measures, GMV and lesion volume differed between CI and CP patients, whereas WMV did not. All brain volumes were reduced in all MS groups compared to controls.

Cognitive profile of patients with MS. As expected, all cognitive domains were affected in CI patients compared to controls, with the strongest effects in information processing speed (z score = −2.54) and executive functioning (z score = −2.53); followed by working memory (z score = −2.45; table 2). Relative to HCs, CP patients had a worse performance only on information processing speed and working memory, while MCI and CI patients scored lower on all cognitive domains compared to controls, which was also seen for CI compared to CP patients.

**Connectivity of the DMN and FPN.** For the DMN, connectivity with the rest of the brain was increased only in CI patients compared to HCs (p corr = 0.04)
and CP patients ($p_{corr} = 0.004$; figure 1), while within-DMN connectivity was not different between groups. For the FPN, similar results were observed; i.e., increased connectivity with the rest of the brain was seen only in CI patients compared to HCs ($p_{corr} = 0.004$) and CP patients ($p_{corr} = 0.004$; figure 2), while within-FPN connectivity did not differ between groups (figure 3). Connectivity between the DMN and FPN was not different between any of the groups.

Results did not change when negative correlations were included in the analysis or when normalized brain volume was added as a covariate. Additionally, group differences in brain volumes were not mediated by functional

### Table 1 Demographic, clinical, and MRI data of included participants

<table>
<thead>
<tr>
<th></th>
<th>HCs</th>
<th>CP</th>
<th>MCI</th>
<th>CI</th>
<th>Overall p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>96</td>
<td>180</td>
<td>65</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>RRMS/SPMS/PPMS, n</td>
<td>148/21/11</td>
<td>46/6/13</td>
<td>49/26/12$^*$</td>
<td>$&lt;0.01^b$</td>
<td></td>
</tr>
<tr>
<td>Age, y$^d$</td>
<td>6 (10.5)</td>
<td>46 (10.5)</td>
<td>49 (12.2)</td>
<td>51 (10.7)$^d$</td>
<td>$&lt;0.01^*$</td>
</tr>
<tr>
<td>F/M, n</td>
<td>56/40</td>
<td>132/48$^d$</td>
<td>42/23</td>
<td>52/35$^*$</td>
<td>0.04$^b$</td>
</tr>
<tr>
<td>Level of education, y$^f$</td>
<td>6 (1–7)</td>
<td>6 (1–7)</td>
<td>4 (1–7)$^d$</td>
<td>4 (2–7)$^d$</td>
<td>$&lt;0.01^a$</td>
</tr>
<tr>
<td>Symptom duration, y$^f$</td>
<td>10 (5–34)</td>
<td>13 (5–35)</td>
<td>18 (5–46)$^a$</td>
<td>0.01$^d$</td>
<td></td>
</tr>
<tr>
<td>EDSS score$^f$</td>
<td>3 (0–8)</td>
<td>3 (0–8)</td>
<td>4 (2–8)$^a$</td>
<td>0.01$^d$</td>
<td></td>
</tr>
<tr>
<td>NBV, L$^c$</td>
<td>1.52 (0.07)</td>
<td>1.48 (0.06)$^d$</td>
<td>1.46 (0.07)$^d$</td>
<td>1.40 (0.09)$^{a,d}$</td>
<td>$&lt;0.01^b$</td>
</tr>
<tr>
<td>NGMV, L$^c$</td>
<td>0.82 (0.05)</td>
<td>0.80 (0.05)$^d$</td>
<td>0.79 (0.05)$^d$</td>
<td>0.75 (0.06)$^{a,d}$</td>
<td>$&lt;0.01^c$</td>
</tr>
<tr>
<td>NDGMV, mL$^c$</td>
<td>62.9 (3.7)</td>
<td>58.6 (5.2)$^d$</td>
<td>56.2 (5.8)$^d$</td>
<td>50.9 (7.8)$^{a,d}$</td>
<td>$&lt;0.01^d$</td>
</tr>
<tr>
<td>NWMV, L$^c$</td>
<td>0.70 (0.03)</td>
<td>0.68 (0.03)$^d$</td>
<td>0.67 (0.03)$^d$</td>
<td>0.65 (0.04)$^d$</td>
<td>$&lt;0.01^d$</td>
</tr>
<tr>
<td>Normalized total lesion load, mL$^c$</td>
<td>10.8 (0.8–83.5)</td>
<td>14.3 (1.8–56.9)</td>
<td>24.0 (2–94.7)$^a$</td>
<td>$&lt;0.01^c$</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI = cognitively impaired; CP = cognitively preserved; EDSS = Expanded Disease Severity Scale; HC = healthy control; MCI = mildly cognitively impaired; NBV = normalized brain volume; NDGMV = normalized deep gray matter volume; NGMV = normalized gray matter volume; NWMV = normalized white matter volume; PPMS = primary progressive multiple sclerosis; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis. For the statistical tests conducted to obtain p values, all assumptions were met.

$^a$ Difference between CI and CP.  
$^b$ The $\chi^2$ test.  
$^c$ Results are expressed as mean (SD) for normally distributed variables.  
$^d$ Difference with HCs.  
$^e$ Analysis of variance.  
$^f$ Results are expressed as median (range).  
$^g$ Kruskal-Wallis test.  
$^h$ Welch test.

### Table 2 Cognitive profile of CI and CP patients

<table>
<thead>
<tr>
<th></th>
<th>HCs</th>
<th>CP</th>
<th>MCI</th>
<th>CI</th>
<th>Overall p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executive functioning</td>
<td>0.00 (0.73)</td>
<td>−0.20 (0.75)</td>
<td>−1.10 (1.02)$^a$</td>
<td>−2.72 (2.21)$^{b,c}$</td>
<td>$&lt;0.001^c$</td>
</tr>
<tr>
<td>Verbal memory</td>
<td>0.00 (0.90)</td>
<td>0.02 (0.88)</td>
<td>−0.56 (1.01)$^a$</td>
<td>−1.48 (1.05)$^{b,c}$</td>
<td>$&lt;0.001^d$</td>
</tr>
<tr>
<td>Information processing speed</td>
<td>0.00 (1.00)</td>
<td>−0.36 (0.94)$^a$</td>
<td>−1.59 (0.68)$^b$</td>
<td>−2.57 (1.17)$^c$</td>
<td>$&lt;0.001^c$</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>0.00 (1.00)</td>
<td>−0.05 (0.95)</td>
<td>−0.74 (0.80)$^a$</td>
<td>−1.16 (0.96)$^{b,c}$</td>
<td>$&lt;0.001^d$</td>
</tr>
<tr>
<td>Visuospatial memory</td>
<td>0.00 (0.94)</td>
<td>−0.17 (1.01)</td>
<td>−0.82 (1.05)$^a$</td>
<td>−1.40 (1.11)$^{b,c}$</td>
<td>$&lt;0.001^d$</td>
</tr>
<tr>
<td>Working memory</td>
<td>0.00 (0.85)</td>
<td>−0.39 (0.79)$^a$</td>
<td>−1.22 (1.01)$^a$</td>
<td>−2.45 (1.94)$^{b,c}$</td>
<td>$&lt;0.001^c$</td>
</tr>
<tr>
<td>Attention</td>
<td>0.00 (0.66)</td>
<td>−0.18 (0.65)</td>
<td>−0.98 (1.05)$^a$</td>
<td>−1.45 (1.50)$^{b,c}$</td>
<td>$&lt;0.001^c$</td>
</tr>
</tbody>
</table>

Abbreviations: CI = cognitively impaired; CP = cognitively preserved; HC = healthy control; MCI = mildly cognitively impaired.  
Mean z scores were obtained with cognitive scores of HCs used as reference. A general linear model or Welch test was used to assess group differences in cognitive performance. Bonferroni-corrected p values are reported.  
$^a$ Difference with HCs.  
$^b$ Difference between CI and CP.  
$^c$ Welch test.  
$^d$ Linear model.
connectivity. Although group effects were observed for the EDSS groups, post hoc comparisons did not survive multiple-comparison correction.

Post hoc regional connectivity changes. Post hoc analysis revealed increased connectivity with the rest of the brain for the inferior parietal ($p_{corr} = 0.01$), posterior cingulate ($p_{corr} = 0.03$), and angular gyri ($p_{corr} = 0.01$). These changes were observed only in CI patients, in whom increased connectivity was observed for the posterior cingulate and angular gyri with the rest of the brain relative to CP ($p_{corr} = 0.04$ and $p_{corr} = 0.004$, respectively) and for the inferior parietal gyrus relative to HCs ($p_{corr} < 0.008$).

Correlates of DMN and FPN connectivity. In MS, increased connectivity scores with the rest of the brain correlated only with worse cognition, including information processing speed and working memory for both the DMN-brain ($r = −0.179$ and $r = −0.171$, respectively) and FPN-brain ($r = −0.176$ and $r = −0.175$, respectively) and attention ($r = −0.152$) only for the FPN-brain. Lower normalized deep GMV was associated with higher connectivity with the rest of the brain of both the DMN ($r = −0.184$) and FPN ($r = −0.149$). Higher EDSS scores were associated with higher connectivity between the DMN and the rest of the brain ($r = 0.142$) (all $p_{corr} < 0.05$; table e-1).

DISCUSSION In this study, we examined the cognitive relevance of the hub-rich and cognitively important DMN and FPN in a large cohort of patients with MS separated into groups of different severities of cognitive impairment. Of the 332 patients, 46% were defined as either MCI or CI, involving multiple cognitive domains, with executive function and information processing speed being most severely affected. Despite such a high incidence in MS and a large body of literature investigating cognitive dysfunction, the specific network properties that drive cognitive deterioration in MS remain unclear. We specifically investigated hub network functioning by separately assessing connectivity within each network, of each network with the rest of the brain, and between the 2 networks. Our results showed that these 2 networks remained internally unchanged in terms of within- and between-network connectivity but showed increased connectivity with the rest of the brain in patients with more severe cognitive impairment.

Regionally, we observed that connectivity increases of the DMN and FPN with the rest of the brain were driven mostly by the inferior parietal, posterior cingulate, and angular cortices. Previous studies have also highlighted the cognitive relevance of structural and functional changes, especially the posterior cingulate gyrus, the most strongly connected hub in the brain. Properties of the human brain network facilitate efficient communication between separate brain regions by strong local connectivity, enabling local processing, and efficient long-distance connections, enabling integration of information. Because hubs are thought to guide most of this information flow in brain networks, they are likely to relate to cognitive function. In light of this perspective, our findings might indicate that especially the functional integration between hub regions and more provincial regions (e.g., non-DMN and non-FPN regions) has been altered.
in CI patients with MS. GM and WM damage throughout the brain may result in a lowered potential for local information processing, forcing the processing load away from peripheral areas toward more centrally located hubs, potentially inducing an inefficient network balance.27 The functional and structural properties of these hub regions might therefore predispose them for pathology, more so than nonhub regions.36 Interestingly, hubs seem to be especially sensitive to such a network pathology across many different neurologic disorders, stressing the clinical relevance of normal hub functioning.28–30 To ensure comparison with other studies, we defined the hub-rich DMN and FPN on the basis of an anatomic atlas. However, future studies are needed to select hub and nonhub regions in a fully data driven way.

Our finding is supported by previous studies in which increased connectivity was most often associated with worse cognitive performance in MS, possibly indicating a maladaptive process.1,2,25 However, these findings seem to be in contrast to the functional reorganization hypothesis in which it would be expected that patients with more severe structural damage would show decreased functional connectivity.14 The histopathologic correlate of the observed increased connectivity remains elusive; however, this could include an abnormally low inhibitory, GABA-based activity of interneurons. In MS, there seems to be a reduction in the population of interneurons,31,32 although it is unclear whether specifically interneurons are more sensitive to MS pathology. Nevertheless, a decrease in GABAergic neurotransmission has been observed in MS.33 In fact, previous neural mass model-based studies have shown that even a small loss of interneurons, and thus a small drop in inhibitory activity, can induce a very large functional effect on hubs and the entire brain network.34 However, given that excitatory neurons are most likely also affected by MS, it seems that a lower inhibitory activity could not be the only mechanism that underlies functional brain changes. For instance, a late and possibly ineffective attempt at beneficial plasticity could present itself by an outreach of hub-rich networks such as the DMN and FPN toward nonhub areas to facilitate information processing and transfer between hub and nonhub regions. Unfortunately, the cross-sectional design of almost all studies in the literature limits strong causal inferences, highlighting the need for longitudinal data.

In contrast to previous studies, we found no changes in within-network connectivity.10,13,35,36 Those studies, however, have been limited mostly to more conventional methodologies and usually do not separate CI and CP patient groups. Previous studies on pediatric and adult MS showed a cognitively relevant increased connectivity of the DMN with other brain networks, but not with the FPN specifically.15,16 Together, these studies seem to support our findings of increased RSN connectivity with other, nonhub, brain networks and no between-network changes.

Exploring the underlying mechanisms of cognitive deficits could help to define whether patients are genuinely CP or CI. Our data support a more conservative criterion of −2 SD on at least 2 tests because no network changes were observed in the MCI group. The normal functioning of both the DMN and FPN is known to be important for a broad range of cognitive demands.4 The increased connectivity of FPN with the rest of the brain was associated mostly with attention deficits in our sample, and the FPN has been implicated as a source of attention control before.23,37 Interestingly, given the involvement of frontal regions, the lack of a relation with our executive function domain is striking. This might be explained by the fact that the concept-shifting task, which makes up the executive functioning domain in our study, also has a prominent information processing speed component. The DMN is usually considered to be engaged during unconstrained cognitive processes, but its relevance for working memory has been shown before.38 Furthermore, functional connectivity changes of the DMN and FPN correlated with normalized deep GMV, the main structural correlate of cognitive dysfunction in MS.2 In addition, the main descriptive predictor of cognitive dysfunction, a lower level of education, was confirmed in the present study, which supports the notion of a cognitive reserve in MS.

We showed that functional connectivity levels of the DMN and FPN can distinguish cognitive phenotypes in MS. In CI patients with MS, these hub-rich networks show an increased level of connectivity, but only with peripheral, nonhub regions of the brain. This seemingly negative change in network balance needs to be investigated further in future longitudinal studies.

AUTHOR CONTRIBUTIONS

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DISCLOSURE

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