

Resting state networks change in clinically isolated syndrome

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Task-functional magnetic resonance imaging studies have shown that early cortical recruitment exists in multiple sclerosis, which can partly explain the discrepancy between conventional magnetic resonance imaging and clinical disability. The study of the brain 'at rest' may provide additional information, because task-induced metabolic changes are relatively small compared to the energy use of the resting brain. We therefore questioned whether functional changes exist at rest in the early phase of multiple sclerosis, and addressed this question by a network analysis of no-task functional magnetic resonance imaging data. Fourteen patients with symptoms suggestive of multiple sclerosis (clinically isolated syndrome), 31 patients with relapsing remitting multiple sclerosis and 41 healthy controls were included. Resting state functional magnetic resonance imaging data were brought to standard space using non-linear registration, and further analysed using multi-subject independent component analysis and individual time-course regression. Eight meaningful resting state networks were identified in our subjects and compared between the three groups with non-parametric permutation testing, using threshold-free cluster enhancement to correct for multiple comparisons. Additionally, quantitative measures of structural damage were obtained. Grey and white matter volumes, normalized for head size, were measured for each subject. White matter integrity was investigated with diffusion tensor measures that were compared between groups voxel-wise using tract-based spatial statistics. Patients with clinically isolated syndrome showed increased synchronization in six of the eight resting state networks, including the default mode network and sensorimotor network, compared to controls or relapsing remitting patients. No significant decreases were found in patients with clinically isolated syndrome. No significant resting state synchronization differences were found between relapsing remitting patients and controls. Normalized grey matter volume was decreased and white matter diffusivity measures were abnormal in relapsing remitting patients compared to controls, whereas no atrophy or diffusivity changes were found for the clinically isolated syndrome group. Thus, early synchronization changes are found in patients with clinically isolated syndrome that are suggestive of cortical reorganization of resting state networks. These changes are lost in patients with relapsing remitting multiple sclerosis with increasing brain damage, indicating that cortical reorganization of resting state networks is an early and finite phenomenon in multiple sclerosis.

Keywords: multiple sclerosis; resting state networks; functional magnetic resonance imaging; independent component analysis; disease progression

Abbreviations: FA = fractional anisotropy; fMRI = functional magnetic resonance imaging; MPRAGE = magnetization prepared rapid acquisition gradient echo; EDSS = expanded disability status scale

Introduction

Multiple sclerosis is an inflammatory, demyelinating disease of the central nervous system, which is commonly diagnosed in the prime of life and in most cases leads to chronic disability. The poor correlation between conventional MRI measures and clinical disability has been improved by the use of quantitative MRI techniques, such as diffusion tensor imaging, magnetization transfer ratio and measures of atrophy, which are more sensitive than conventional MRI for damage that can be found in the grey matter and white matter outside focal white matter lesions (Barkhof, 2002). Quantitative MRI may also measure more specifically the pathological process of neurodegeneration that occurs in multiple sclerosis (Trapp *et al.*, 1998) and is thought to be largely responsible for clinical progression (Bjartmar *et al.*, 2003).

The clinical-MRI discrepancy can be bridged further by investigating adaptive mechanisms in multiple sclerosis, such as recruitment of other brain areas (cortical reorganization), that occur in the brain in reaction to damage. As has been illustrated using motor and cognitive tasks during the acquisition of functional MRI (fMRI), regional cortical reorganization is present in multiple sclerosis throughout the disease course (Rocca *et al.*, 2005), including patients with very short disease duration (Audoin *et al.*, 2003).

It has recently been shown that task-related increases of neuronal metabolism are small, less than 5% compared to the metabolism of the brain at rest (Raichle and Mintun, 2006). Because task-fMRI in multiple sclerosis may thus only reveal the proverbial tip of the iceberg, it is critical to take into account brain activity that occurs in the absence of external stimulation in order to understand better how the brain functions and adapts in patients with multiple sclerosis. Spontaneous low-frequency fluctuations of the cerebral blood oxygenation level-dependent signal can be measured during rest (no task) with fMRI (Lowe *et al.*, 2000). These fluctuations have shown strong temporal coherence between brain regions (synchronization) that represent functional systems, or so-called resting state networks, engaged in e.g. sensorimotor, attention and visual processing (Greicius *et al.*, 2003; Damoiseaux *et al.*, 2006). New data-driven analysis techniques have been developed, such as independent component analysis, that are well-suited for the analysis of fMRI data in the absence of an active task paradigm. A recent supplement to group-independent component analysis, called dual regression (Beckmann *et al.*, 2009; Filippini *et al.*, 2009), allows for a principled comparison between groups, using second level spatial correlations based on individual time-courses.

Resting state synchronization was found to be reduced in Alzheimer's disease (Greicius *et al.*, 2004). Altered resting state synchronization has also been found in schizophrenia (Liu *et al.*, 2008) and depression (Greicius *et al.*, 2007). Early work using correlation analysis revealed reduced resting state synchronization between left and right motor cortex in patients with multiple

sclerosis (Lowe *et al.*, 2002). How other resting state networks are affected by multiple sclerosis has not yet been shown. Based on the finding in other neurological diseases that cortical reorganization of resting state networks already exist in early patients, such as in patients with early Alzheimer's disease (Sorg *et al.*, 2007; Wang *et al.*, 2007), we hypothesized that in multiple sclerosis resting state networks may also be modified early in the disease course. In our study we therefore compared resting state networks between patients with clinically isolated syndrome, relapsing remitting multiple sclerosis patients and healthy controls. This intermediate phenotype was put into perspective by determining the degree of structural damage and clinical function.

Materials and methods

Subjects

Forty-five patients were selected from a clinical multiple sclerosis database from our multiple sclerosis centre and included in the current study. Mean age of the patients was 37.7 (SD: 9.0) years and 33 of them were female. Fourteen out of these patients had clinically isolated syndrome and median disease duration from onset of 1.4 years (interquartile range: 0.8–1.5). The remaining 31 patients had a diagnosis of clinically definite multiple sclerosis (Polman *et al.*, 2005) and a relapsing remitting disease type (Lublin and Reingold, 1996), with a median disease duration of 3.5 years (interquartile range: 1.1–8.5). Forty-one age- and sex-matched healthy controls [mean age 38.6 (SD: 10.5) years; 27 female], free of neurological or psychiatric disorders, were also included. The study protocol was approved by the institutional ethics review board and all subjects gave written informed consent prior to participation.

Physical disability was assessed in patients by the expanded disability status scale (EDSS) (Kurtzke, 1983) on the day of scanning and all subjects underwent neuropsychological screening. This consisted of three tasks for the domains most frequently impaired in multiple sclerosis (Chiaravalloti and DeLuca, 2008): memory, attention and inhibition, and processing speed. Spatial memory was assessed using the Location Learning Test (Bucks and Willison, 1997). Attention and inhibition were assessed using the Stroop Interference Test (Stroop, 1935). The Letter Digit Substitution Test (Jolles *et al.*, 1995) was included to assess processing speed of visual information.

In order to characterize our subjects further, symptoms indicative of depression and anxiety were assessed by the Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983). Fatigue was assessed by the Checklist of Individual Strength questionnaire (Vercoulen *et al.*, 1994). Pre-morbid intelligence was measured using the Dutch version of the New Adult Reading Test (Nelson and O'Connell, 1978; Schmand *et al.*, 1991). Lastly, handedness was evaluated in all subjects using the Edinburgh Handedness Scale (Oldfield, 1971).

MRI

MRI was performed on a 1.5T whole-body magnetic resonance system (Siemens Sonata, Erlangen, Germany), using an eight-channel phased-array head coil. Single-slab T₁-weighted magnetization

prepared rapid acquisition gradient echo (MPRAGE) images (repetition time = 2700 ms, echo time = 5 ms, inversion time = 950 ms; 176 sagittal partitions with 1.3 mm thickness; $248 \times 330 \text{ mm}^2$ field of view and $1.3 \times 1.3 \text{ mm}^2$ in-plane resolution; acquisition time = 4.9 min) were obtained for volumetric and registration purposes, using parallel imaging and an acceleration factor of 2. For fMRI during resting state, 200 volumes of echo planar images were acquired (repetition time = 2850 ms, echo time = 60 ms; 36 axial slices with isotropic 3.3-mm voxels and $211 \times 211 \text{ mm}^2$ field of view; acquisition time = 9.5 min). Subjects were instructed to rest with their eyes closed, not fall asleep, and think of nothing in particular during this scan. Diffusion-weighted echo planar images (repetition time = 8500 ms, echo time = 86 ms; 59 contiguous axial slices with an isotropic 2 mm resolution; acquisition time = 10 min) with 60 volumes with non-collinear diffusion gradients (b -value of 700 s/mm^2) and 10 volumes without diffusion weighting were also acquired. Furthermore, for lesion information interleaved turbo spin-echo proton density and T_2 -weighted images (repetition time = 3130 ms, echo time = 24/85 ms; 46 3-mm-thick axial slices; $192 \times 256 \text{ mm}^2$ field of view and $1.0 \times 1.0 \text{ mm}^2$ in-plane resolution) were obtained, as well as spin-echo T_1 -weighted images (repetition time = 485 ms, echo time = 12 ms, 46 axial 3-mm-thick slices; $192 \times 256 \text{ mm}^2$ field of view and $1.0 \times 1.0 \text{ mm}^2$ in-plane resolution).

fMRI

All image manipulation tools used in this study are part of FMRIB's Software Library (<http://www.fmrib.ox.ac.uk/fsl>). The following pre-statistics processing was applied. For each subject, the first two (of 200) volumes of functional magnetic resonance images were removed to analyse only those in which steady-state imaging had been reached. Next, the functional magnetic resonance images were motion corrected and non-brain tissue was removed. The images were spatially smoothed using a Gaussian kernel of 5 mm full width at half maximum. 4D grand-mean scaling was performed in order to ensure comparability between data sets at the group level. In addition, high-pass temporal filtering (Gaussian-weighted least-squares straight fitting line fitting, using a cut-off of 100.0 s) was applied. After this pre-processing the functional scan was aligned to the subject's high resolution T_1 -weighted image, and subsequently to Montreal Neurological Institute-152 standard space using non-linear registration (warp resolution: 10 mm). The aligned data were then temporally concatenated across subjects to create a single 4D data set.

This concatenated fMRI data set was decomposed using independent component analysis (Beckmann *et al.*, 2005) to identify large-scale patterns of functional connectivity. In this analysis, the data set was decomposed into 21 components; this model order was estimated using the Laplace approximation to the Bayesian evidence for a probabilistic principal component model.

Next, the between-subject analysis of the resting data was carried out using a 'dual-regression' approach (Beckmann *et al.*, 2009) that allows for voxel-wise comparisons of resting functional connectivity. Within each subject's fMRI dataset, subject-specific temporal dynamics and associated spatial maps were identified. This involves using the full set of group-independent component analysis spatial maps in a linear model fit (spatial regression) against the separate subject's fMRI data sets, resulting in matrices describing temporal dynamics for each component and subject. Then, these time-course matrices were variance-normalized and used in a linear model fit (temporal regression) against the associated subject's fMRI data set to estimate subject-specific spatial maps. We refer below to these maps as reflecting 'synchronization' across resting-state networks; however, this is

more than just a coherence measure (which implies independence of amplitude). Because of the normalization of the variance of the time series used in the final regression, these spatial maps reflect both amplitude of spontaneous fluctuation in a network, as well as its coherence across space.

Finally, the different component maps were collected across subjects into single 4D files for each original independent component analysis map. Components of interest were selected by visual inspection and comparison to earlier studies (Beckmann *et al.*, 2005). These were tested voxel-wise for statistically significant differences between our groups with a general linear model, correcting for age and sex, using nonparametric permutation testing (5000 permutations). Summary head movement parameters were separately added as covariates to investigate their possible confounding effect. The generated spatial maps characterizing between-group differences were controlled for multiple comparisons with a corrected threshold of $P < 0.05$, using threshold-free cluster enhancement (Smith and Nichols, 2009).

Brain volume and lesion volume measurements

Brain volume, normalized for head size, was measured using the MPRAGE images and automated segmentation-based estimation software (Smith *et al.*, 2002). Thus normalized grey matter volume and white matter volume were also determined. Lesions were marked and manually outlined on the proton density and T_1 -weighted images using a local-threshold technique (ALICE software; Perceptive Informatics). Subsequently, T_2 hyperintense and T_1 hypointense lesion volumes were calculated for each patient.

White matter tract analysis

Motion and eddy current distortion of the diffusion-weighted images were corrected with FMRIB's Diffusion Toolbox, which was also used to fit the diffusion tensor for each voxel. From this tensor, fractional anisotropy (FA) was derived voxelwise and analysed using tract-based spatial statistics (Smith *et al.*, 2006). Individual FA images were non-linearly registered to the FMRIB58 FA standard-space image. The FA images in standard space were averaged and the result, a mean FA image, was skeletonized. The resulting mean FA skeleton image was thresholded at 0.2 to include only white matter. For each subject's registered FA image, the maximum FA value perpendicular to each voxel of the skeleton was projected onto this skeleton. Differences between patients with clinically isolated syndrome, relapsing remitting patients and controls were analysed in a voxelwise fashion, correcting for age and sex and controlled for multiple comparisons, again using permutation inference statistics and threshold-free cluster enhancement at corrected $P < 0.05$.

Statistical analyses

All statistical analyses in this study, other than the statistical analyses included in the MRI analysis tools, were performed using Statistical Package for the Social Sciences version 16.0 (SPSS, Chicago, IL, USA). Demographic, clinical and volumetric MRI data were statistically compared between the groups using student's t -test when data were normally distributed, otherwise using the Mann-Whitney U -test. The Fisher's exact test was used to evaluate possible differences in proportions.

Relations between synchronization values extracted from areas of difference on the one hand, and structural MRI measures (normalized

brain, grey matter and white matter volumes, white matter skeleton FA, lesion volumes) and clinical measures (EDSS and cognitive test results) on the other hand, were assessed in the patient groups using Spearman's rank correlation coefficient. *P*-values <0.05 were considered statistically significant.

Results

Subject descriptives

Table 1 presents the demographical and clinical data of our subjects. As expected, median EDSS and disease duration for patients with clinically isolated syndrome were lower than those of relapsing remitting patients. Patients with clinically isolated syndrome originally presented at first clinical episode with the following symptoms: two patients with optic neuritis; six patients with long-tract sensory symptoms; two patients with long-tract motor symptoms; two patients with coordination/balance problems; one patient with dysarthria; and lastly one patient with multifocal symptoms. Eleven of our patients with clinically isolated syndrome showed lesions in the brain or spinal cord, and four of them fulfilled the MRI criterion of dissemination in time. The MRI criterion for dissemination in space was fulfilled by one patient. Nine of the patients with clinically isolated syndrome have been followed clinically after their participation in the current study a median 2.5 years after presenting symptoms; three of them were at that time clinically converted. Mean premorbid IQ of controls, calculated from the Adult Reading Test, did not differ significantly from that of clinically isolated syndrome or relapsing remitting patients. Cognitive performance of patients with clinically isolated syndrome did not differ significantly from that of controls, although a trend was found for the Stroop Test. Neither did patients with clinically isolated syndrome differ from controls in the fatigue, anxiety and

depression measures. In contrast, relapsing remitting patients showed significantly decreased attention and processing speed compared to controls, as well as increased fatigue, anxiety and depression scores. Based on the Edinburgh Handedness Scale, 36 (88%) controls, 12 (86%) clinically isolated syndrome and 27 (87%) relapsing remitting patients were right-handed.

Resting state network analysis

Twenty-one components were computed in the entire subject group by independent component analysis. Eight of these components coincided with resting state networks described in previous studies (Beckmann and Smith, 2005; Damoiseaux *et al.*, 2006; De Luca *et al.*, 2006). Figure 1 shows these components, denoted previously as reflecting executive functioning (Fig. 1A), sensorimotor function (Fig. 1B), ventral and dorsal attention (Fig. 1C), the default mode network (Fig. 1D), working memory or the left and right frontoparietal networks (Fig. 1E–F), visual processing (Fig. 1G) and, lastly, auditory and language processing (Fig. 1H).

These eight resting state networks were compared between the three groups. Patients with clinically isolated syndrome showed areas of significantly higher synchronization compared to healthy controls (Fig. 1) in the executive function network (left medial prefrontal cortex), the attention system (bilateral precuneus) and the sensorimotor function network (right premotor and sensory cortices). In this last network, sub-threshold ($P < 0.1$) increased synchronization was also found in the left premotor cortex, as well as in the supplementary motor area. Patients with clinically isolated syndrome also showed significantly higher synchronization than relapsing remitting patients in the attention system, also in the precuneus. Furthermore, significantly increased synchronization was found in patients with clinically isolated syndrome compared to relapsing remitting patients in the default mode network (posterior cingulate gyrus), as well as in the left

Table 1 Demographic and clinical measures of controls, patients with clinically isolated syndrome and relapsing remitting patients

	Controls	Clinically isolated syndrome	Relapsing remitting
Number of patients	41	14	31
Mean age (SD) in years	38.6 (10.5)	34.6 (8.4)	39.1 (9.0)
Gender (female/male)	27/14	9/5	24/7
Handedness (R/L/M)	36/3/2	12/1/1	27/2/2
Median disease duration (IQR) in years ^a	–	1.4 (0.8–1.5)	3.5 (1.1–8.5)
Median EDSS (IQR)	–	2.0 (1.0–2.6)	2.5 (2.0–3.5)
Mean IQ (SD)	104 (10)	100 (11)	106 (12)
Median LLT (IQR)	11.0 (4.5–24.5)	13.0 (3.5–20.0)	14.0 (5.0–23.0)
Mean Stroop (SD) ^b	53.6 (10.7)	48.5 (6.0)	48.5 (8.8)
Mean LDMT (SD) ^c	63.7 (9.7)	64.0 (9.5)	58.3 (9.2)
Median HADS-A (IQR) ^d	4.0 (2.0–5.5)	4.5 (3.0–7.5)	5.0 (4.0–8.0)
Median HADS-D (IQR) ^e	1.0 (0–2.0)	2.0 (1.0–3.5)	2.0 (1.0–7.3)
Median CIS-20 (IQR) ^f	34 (22–50)	44 (30–66)	54 (41–84)

SD = standard deviation; IQR = interquartile range; LLT = Location Learning Test; LDMT = Letter Digit Modification Test; HADS = Hospital Anxiety and Depression Scale; CIS-20 = Fatigue Questionnaire.

Significant differences between groups.

a clinically isolated syndrome versus relapsing remitting ($P = 0.01$).

b, c controls versus relapsing remitting ($P < 0.05$).

d, e, f controls versus relapsing remitting ($P < 0.01$).

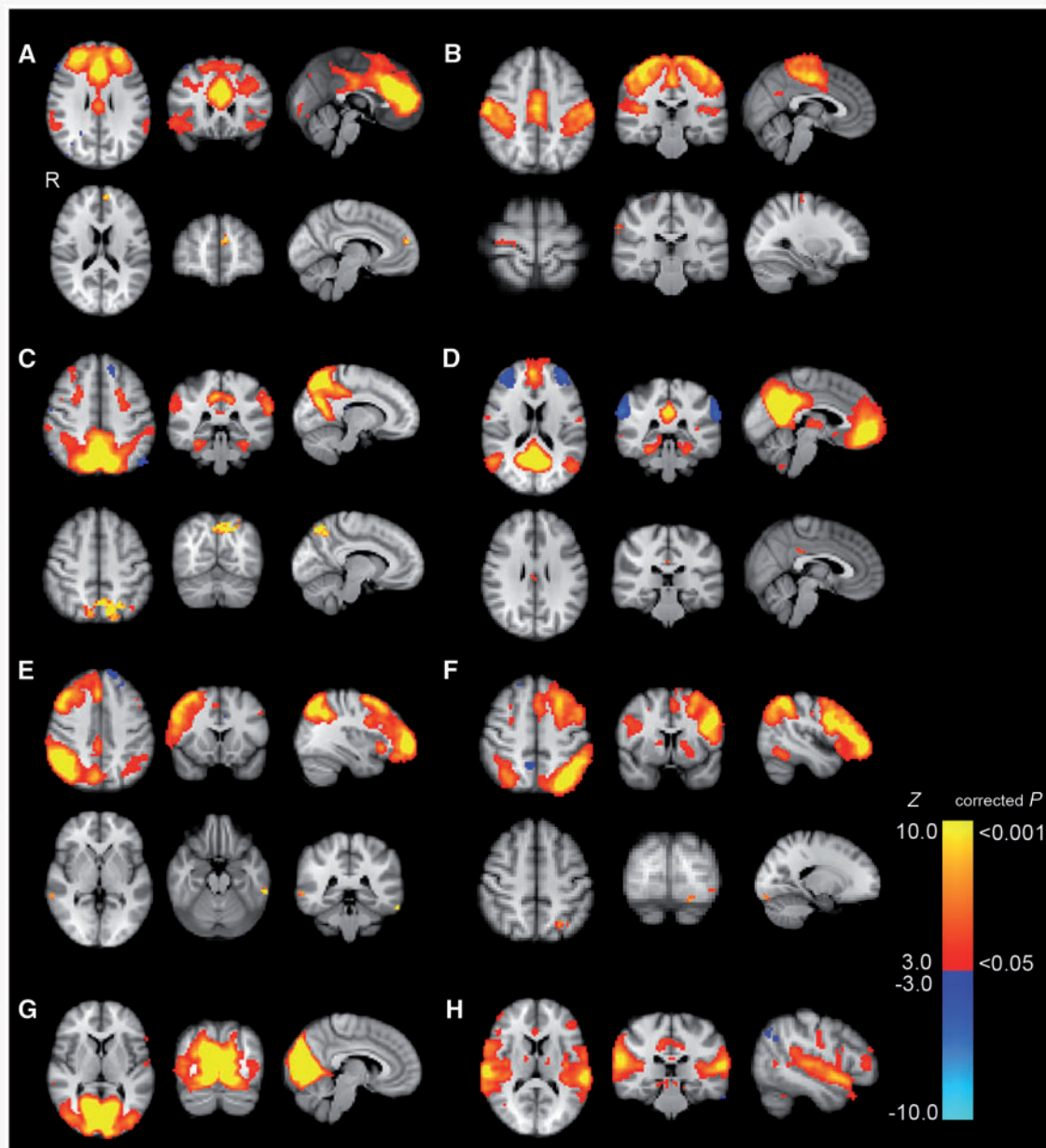


Figure 1 The upper rows show resting state networks identified with independent component analysis; the lower rows show increased synchronization in patients with clinically isolated syndrome for each network. (A) Executive functioning network; synchronization was increased compared to controls in the left medial prefrontal cortex. (B) Sensorimotor network: increased synchronization compared to controls in the right premotor cortex and inferior parietal gyrus. (C) Ventral and dorsal attention system: increased synchronization compared to controls in the bilateral precuneus. Synchronization was also increased compared to relapsing remitting patients in the precuneus. (D) Default mode network: increased synchronization compared to relapsing remitting patients in the posterior cingulate gyrus. (E) Right frontoparietal network: increased synchronization compared to relapsing remitting patients in the left inferior temporal gyrus and right superior temporal gyrus. (F) Left frontoparietal network: increased synchronization compared to relapsing remitting patients in the left superior parietal gyrus and the occipital lobe. (G) Visual processing and (H) auditory and language processing: no significant differences between groups were found in these networks.

frontoparietal network (left superior parietal gyrus) and the right frontoparietal network (left inferior temporal gyrus and right superior temporal gyrus). Thus, areas of increased synchronization were present in patients with clinically isolated syndrome compared to controls or relapsing remitting patients in six out of the eight total common resting state networks. Areas of significantly

decreased synchronization in patients with clinically isolated syndrome compared to controls or relapsing remitting patients were not found. No significant increased or decreased synchronization in the resting state networks were found in relapsing remitting patients compared to healthy controls. The mean synchronization values from areas of difference between the groups are reported

in Table 2, which shows that differences that were found in clinically isolated syndrome patients compared to controls were lost in relapsing remitting patients.

Mean 'relative' (timepoint-to-timepoint) head motion during the acquisition of the fMRI did not differ significantly between the groups, although mean 'absolute' displacement (a less important metric of motion than the 'relative' measure) was significantly higher in clinically isolated syndrome patients (0.34 mm, SD 0.22) compared to the other two groups (controls: 0.23 mm, SD 0.14; relapsing remitting: 0.19 mm, SD 0.14). Adding absolute mean displacement for each subject as a covariate in the final between-group analysis did not alter any of the results mentioned above.

Brain and lesion volumes

In Table 3, results from the structural MRI analysis are provided. T₂ hyperintense and T₁ hypointense lesion volumes were significantly lower in patients with clinically isolated syndrome compared to relapsing remitting patients. In relapsing remitting patients, normalized brain volume (1.58 l, SD 0.08) was significantly lower than in controls (1.63 l, SD 0.06; $P=0.01$) and in patients with clinically isolated syndrome ($P<0.05$). This mainly resulted from a lower mean normalized grey matter volume in relapsing remitting patients (0.94 l, SD 0.06) compared to controls (0.97 l, SD 0.06; $P=0.02$) or patients with clinically isolated syndrome (0.98 l, SD 0.06; $P<0.05$). Parenchymal volumes of patients with clinically

isolated syndrome were not significantly different from those of healthy controls.

Mean FA is provided for each group in Table 3. No areas of significant differences of FA were found between patients with clinically isolated syndrome and healthy controls. FA was decreased in large areas of the white matter (beyond focal lesions) in relapsing remitting patients compared to healthy controls, as well as compared to patients with clinically isolated syndrome. For each group, FA values of areas of differences are anatomically listed in the online Supplementary Table.

Relation between functional and structural measures

No significant relations were found in the clinically isolated syndrome or in the relapsing remitting patient group between the synchronization values extracted from areas of difference on the one hand, and structural MRI measures (normalized brain, white matter and grey matter volumes, white matter skeleton FA, lesion volumes) and clinical measures (EDSS, cognitive tests) on the other hand. However, in the entire multiple sclerosis patient group, synchronization values from several networks were found to decrease gradually with increasing damage; these correlations were significant between white matter skeleton FA and synchronization values from the default mode network (Spearman's rho 0.34; $P=0.02$) and from the ventral and dorsal attention system

Table 2 Synchronization values (Z-scores) extracted from areas of significantly increased synchronization in patients with clinically isolated syndrome for each network

	Controls		Clinically isolated syndrome		Relapsing remitting	
	median	IQR	median	IQR	median	IQR
Executive function (Fig. 1A)	20.8	14.4–27.0	32.7	26.1–48.9	21.9	13.5–29.7
Sensorimotor function (Fig. 1B)	26.0	19.3–36.2	37.0	25.7–92.1	25.4	22.6–37.7
Attention system (Fig. 1C)	43.2	32.7–51.9	49.0	36.5–104.2	43.0	33.1–51.4
Default mode network (Fig. 1D)	30.0	21.7–40.9	40.8	33.9–59.7	24.7	18.6–36.4
Frontoparietal right (Fig. 1E)	11.1	5.6–17.6	23.1	16.5–30.4	6.0	2.3–8.4
Frontoparietal left (Fig. 1F)	5.9	3.5–9.2	14.9	8.7–37.9	4.8	0.6–7.6

IQR = interquartile range.

Table 3 Structural MRI measures of controls, patients with clinically isolated syndrome and relapsing remitting patients

	Controls	Clinically isolated syndrome	Relapsing remitting
Mean normalized brain volume (SD) in l ^a	1.63 (0.06)	1.63 (0.07)	1.58 (0.08)
Mean normalized grey matter volume (SD) in l ^b	0.97 (0.05)	0.98 (0.06)	0.94 (0.06)
Mean normalized white matter volume (SD) in l	0.66 (0.04)	0.65 (0.03)	0.65 (0.05)
Median T ₂ lesion volume (IQR) in ml ^c	–	0.1 (0–1.1)	1.8 (0.7–3.1)
Median T ₁ hypointense lesion volume (IQR) in ml ^d	–	0 (0–0.2)	0.5 (0.2–1.2)
Mean FA of the white matter skeleton (SD) ^e	0.294 (0.01)	0.297 (0.01)	0.285 (0.01)

SD = standard deviation; IQR = interquartile range.

Significant differences between groups:

a clinically isolated syndrome versus relapsing remitting ($P=0.05$), controls versus relapsing remitting ($P=0.01$).

b relapsing remitting versus clinically isolated syndrome and versus controls ($P<0.05$); clinically isolated syndrome versus relapsing remitting ($P<0.01$).

c, d clinically isolated syndrome versus relapsing remitting ($P<0.01$).

e relapsing remitting versus clinically isolated syndrome and versus controls ($P<0.01$).

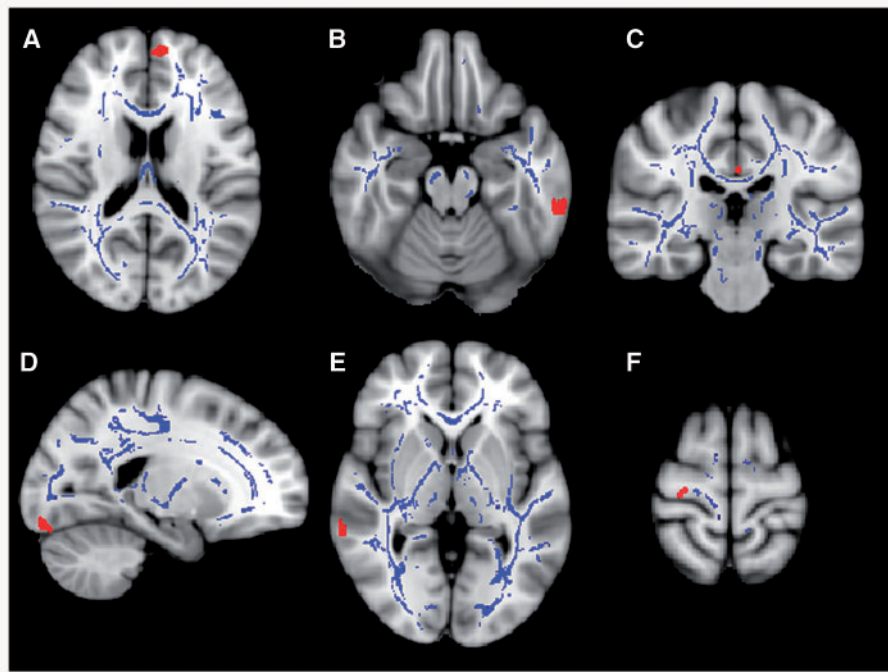


Figure 2 A qualitative spatial relation, i.e. anatomical correspondence with an adjacent white matter tract, was found for eight of the nine areas of increased synchronization. Examples are shown in this figure, with areas of decreased FA in relapsing remitting patients in blue and increased synchronization found in clinically isolated syndrome patients in red. The background image is a mean 3D T₁ image. (A) Left medial prefrontal cortex, part of executive functioning network. (B) Left inferior temporal gyrus, part of right frontoparietal network; for this area a significant quantitative relation was found in addition to the anatomical correspondence. (C) Posterior cingulate gyrus, part of default mode network. (D) Left occipital lobe, part of left frontoparietal network. (E) Right superior temporal gyrus, part of the right frontoparietal network. (F) Premotor cortex, part of the sensorimotor network; for this area a significant quantitative relation was found as well.

(rho 0.29; $P=0.05$). To test the hypothesis that the increased synchronization disappears when local damage in nearby white matter tracts occurs, an overlay was made of the resting state network differences and the white matter FA changes. Qualitative spatial relations, i.e. anatomical correspondence with an adjacent white matter tract, were found for eight of the nine areas of increased synchronization. Examples are shown in Fig. 2. Only for increased activation in the left superior parietal gyrus of the left frontoparietal network, anatomical correspondence was not found. In a subsequent quantitative analysis the mean FA of the white matter tract was extracted and related to synchronization values of areas of increases. Significant relations with FA reductions were found in the total patient group for the increased synchronization in the left inferior temporal gyrus, part of the right frontoparietal network (Spearman's rho 0.38; $P<0.01$) and for the increased synchronization in the right premotor cortex, part of the sensorimotor network (rho 0.30; $P=0.05$).

Discussion

Several network-specific increases of synchronization were found in clinically isolated syndrome patients during rest, including increased synchronization in the default mode network. These changes were found in the absence of outspoken cognitive impairment, and may reflect recruitment of reserve capacity

(functional reorganization) to compensate for (limited) damage. Consistent with this hypothesis of early but finite reorganization, resting state network changes were not found in relapsing remitting patients in whom cognitive impairment was indeed present, as well as grey matter atrophy and changes in diffusion measures throughout the white matter.

What is measured when fMRI of the brain is performed in the absence of a task has been discussed in a recent review (Fox and Raichle, 2007). Spontaneous blood oxygenation level-dependent fluctuations have been shown to be specifically correlated between functionally related brain regions and have been linked to known anatomical systems, including systems involved in higher cognitive function (Damoiseaux *et al.*, 2006; van den Heuvel *et al.*, 2009; Smith *et al.*, 2009). Evidence for a neuronal nature of resting state networks comes from high correlations found between spontaneous blood oxygenation level-dependent fluctuations and power in different electroencephalogram bands (Laufs *et al.*, 2003). Resting state networks are an intrinsic property of the brain, as they can be found across different resting states, including sleep (Horowitz *et al.*, 2009) and anaesthesia (Kiviniemi *et al.*, 2003), as well as during tasks (Smith *et al.*, 2009). Advantages of analysing resting state fMRI data with independent component analysis, in comparison to a region of interest based analysis, include a more efficient removal of signals caused by physiological noise and the fact that it is data-driven; no *a priori* hypotheses are necessary, which can yield unexpected results from

the data (Beckmann and Smith, 2005). Because of the relative novelty of resting state research, few studies exist in which resting state fMRI in multiple sclerosis has been investigated. We have therefore compared our results mainly to insights from resting state fMRI studies in healthy controls and to those few studies in which other neurological diseases were investigated.

Initially, resting state studies have focussed on the default mode network, a network found to be more 'active' when the subject is at rest, i.e. deactivating in subjects responding to stimuli. In our study we found that synchronization in the default mode network is increased at rest in the cingulate gyrus in patients with clinically isolated syndrome compared to controls, and that this increase is lost in relapsing remitting patients. This network is thought to monitor the internal and external environment for the detection of relevant events, thereby keeping a background level of attention (Raichle *et al.*, 2001). In healthy controls, the default mode network was recently shown to be more active immediately after cognitively challenging tasks (Pyka *et al.*, 2009). In patients with Alzheimer's disease, and with mild cognitive impairment at risk for Alzheimer's disease, decreased activity within the default mode network has been found consistently (Greicius *et al.*, 2004; Sorg *et al.*, 2007). Interestingly, a recent study, using a similar independent component analysis and dual regression analysis as in our study, reported increased (hippocampal) synchronization in the default mode network in young healthy *APOE-ε4* allele carriers compared to age-matched non-carriers (Filippini *et al.*, 2009). They repeated this finding in the same study with an additional memory encoding task-fMRI, thereby confirming results from a previous positron emission tomography study (Bookheimer *et al.*, 2000).

In addition to the default mode network, other resting state networks that are of interest were also comprehensively investigated in our study. For the sensorimotor network, increases of synchronization compared to controls found in our study were significant in the hemisphere that is non-dominant for the majority of our patients with clinically isolated syndrome, but sub-threshold ($P < 0.1$) increased synchronization was also found in the dominant premotor cortex and in the supplementary motor area. Synchronization of the motor cortex found in healthy controls during rest started interest in the resting brain (Biswal *et al.*, 1995). It was recently found that functional subregions of the motor network are one-on-one linked during rest to their homolog in the contralateral hemisphere and that they are thus organized in a somatotopic fashion (van den Heuvel and Hulshoff Pol, 2009). A region of interest approach study involving patients with multiple sclerosis reported decreased functional synchronization during rest between right- and left-hemisphere primary motor cortices, when compared to controls (Lowe *et al.*, 2002). Interestingly, in a recent independent component analysis study amyotrophic lateral sclerosis patients showed differences in resting state synchronization of the motor network (Mohammadi *et al.*, 2009).

In our study, increased synchronization in patients with clinically isolated syndrome was also found within networks associated with cognitive function—the executive function network (compared to controls) and the dorsal and ventral attention system (compared to controls and to relapsing remitting patients). Additionally, recruitment of brain areas in patients with clinically isolated syndrome

compared to relapsing remitting patients was found beyond the normal boundary of the two lateralized frontoparietal networks, recently associated in healthy controls with inhibition and perception/pain, as well as with language (Smith *et al.*, 2009). Part of the added value of resting state fMRI in future studies could lay in discriminating between increased 'baseline connectivity' and increases of connectivity that are truly task-dependent.

No significant differences between the groups, and especially no increases in patients with clinically isolated syndrome were found for the visual network and the auditory and language processing network. That the activity pattern of networks responsible for such 'basic' functions would not differ between groups could be expected and these results thus generate confidence that the differences found between groups in the other networks are meaningful (Greicius, 2008).

The functional status in patients with multiple sclerosis is considered to be the result of a complex interaction between inflammation, demyelination and axonal damage on the one hand, and the reaction of the brain through repair, remyelination and cortical reorganization on the other hand. In later disease stages, irreversible axonal injury is considered to be largely responsible for clinicognitive impairment (Bjartmar and Trapp, 2001), but axonal damage is probably relatively limited at the time of the first clinical attack (Miller *et al.*, 2005), as indicated by magnetic resonance spectroscopy studies (Fernando *et al.*, 2004). Although our patients with clinically isolated syndrome are typical from a clinical point of view, the findings of normal white matter diffusion measures and absence of significant brain atrophy in this group indicate that they suffered from benign pathology (Raz *et al.*, 2010). Axonal damage is known to accumulate in the first years after disease onset, especially in those patients that convert to multiple sclerosis more rapidly (Dalton *et al.*, 2004; Calabrese *et al.*, 2007; Wattjes *et al.*, 2008). Relapsing remitting patients are known to suffer from significant grey matter atrophy (Fisher *et al.*, 2008), which was the case for the relapsing remitting patients in our study.

Recent neurobiological theories (McIntosh, 2000; Sporns *et al.*, 2004) have emphasized the importance of the interaction between brain regions, rather than the individual activity patterns of these brain regions, in explaining higher (cognitive) brain function. From this standpoint, damage to one brain region may affect several other areas in the brain. A reserve capacity explains why there are few functional deficits in the beginning stages of the disease, but is apparently limited because decreased clinical function develops over time as functional reorganization has run its course.

A possible confounder in the interpretation of fMRI studies arises when a mismatch between patients and controls for task performance contributes to differences in fMRI activation. In the investigation of motor function a solution is the use of passive movement (Ciccarelli *et al.*, 2006), but for cognitive function the confounder of mismatched performance is more difficult to solve. One advantage of resting state fMRI analysis is that differences found cannot be explained by an increased effort in the patient group, and that these differences may thus reflect functional reorganization in a more fundamental manner.

Functional reorganization was probably induced by structural injury. Damage could however not be detected with diffusion

tensor imaging in the white matter of patients with clinically isolated syndrome, and no relations were found with values extracted from areas of increased synchronization. It is unclear whether this indicates that white matter damage can already provoke extensive functional changes when it is still very subtle, or that grey matter damage, more difficult to identify, was responsible. An equally fascinating question is: what precisely causes the resting state increases to eventually disappear in relapsing remitting patients? Future longitudinal studies should investigate these questions with regional measures of structural damage, and also whether a window of opportunity exists during which increased activity of resting state networks is adaptive, and can be possibly extended by pharmacotherapy.

In conclusion, increased synchronization during rest in several specific brain networks is already found at the clinically isolated syndrome stage of multiple sclerosis. This cortical reorganization of resting state networks is an early and finite phenomenon, as brain damage in the grey and white matter have increased in relapsing remitting patients and clinical disability has ensued.

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Supplementary material

Supplementary material is available at *Brain* online.

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