NeuroImage: Clinical 4 (2014) 403–410

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

Graph theoretical analysis indicates cognitive impairment in MS stems from neural disconnection

Jeroen Van Schependom [a,c,*], Jeroen Gielen [a], Jorne Laton [a], Marie B. D'hooghe [a,b], Jacques De Keyser [b], Guy Nagels [a,b,c]

[a] UZ Brussel, Vrije Universiteit Brussel, Center for Neurosciences, Laarbeeklaan 101, 1090 Brussels, Belgium
[b] National MS Center Melsbroek, Vanheylenstraat 16, 1820 Melsbroek, Belgium
[c] Faculté de Psychologie et des Sciences de l'Education, Place du parc 20, 7000 Mons, Belgium

ARTICLE INFO

Article history:
Received 3 December 2013
Received in revised form 14 January 2014
Accepted 22 January 2014
Available online 31 January 2014

Keywords:
Network analysis
Cognitive impairment
Graph theoretical analysis
Connectome
Multiple sclerosis

ABSTRACT

Background: The mechanisms underlying cognitive impairment in MS are still poorly understood. However, due to the specific pathology of MS, one can expect alterations in connectivity leading to physical and cognitive impairment.
Aim: In this study we aimed at assessing connectivity differences in EEG between cognitively impaired (CI) and cognitively preserved (CP) MS patients. We also investigated the influence of the measures used to construct networks.
Methods: We included 308 MS patients and divided them into two groups based on their cognitive score. Graph theoretical network analyses were conducted based on networks constructed using different connectivity measures, i.e. correlation, correlation in the frequency domain, coherence, partial correlation, the phase lag index and the imaginary part of coherency. The most commonly encountered network parameters were calculated and compared between the two groups using Wilcoxon's rank test. Clustering coefficients and path lengths were normalized to a randomized mean clustering coefficient and path length for each patient. False discovery rate was used to correct for the multiple comparisons and Cohen's d effect sizes are reported.
Results: Coherence analysis suggests that theta and delta connectivity is significantly smaller in cognitively impaired patients. Small-worldness differences are found in networks based on correlation, theta and delta coherence and correlation in the frequency domain. Modularity was related to age but not to cognition.
Conclusion: Cognitive deterioration in MS is a symptom that seems to be caused by neural disconnections, probably the white matter tracts connecting both hemispheres, and leads to a wide range in network differences which can be assessed by applying GTA to EEG data. In the future, these results may lead to cheaper and more objective assessments of cognitive impairment in MS.

1. Introduction

In recent years a vast amount of research has been devoted to the study of the human connectome. These efforts have greatly advanced our understanding of the working of the brain in both healthy control groups and disease groups (Sporns, 2011; Sporns et al., 2005). Graph theoretical analysis (GTA) of both functional and structural data has revealed important topological properties such as small-worldness and highly connected ('rich') hub regions (Eguíluz et al., 2005; Shu et al., 2011; Stam et al., 2009).

Multiple Sclerosis (MS) is the most prevalent neurodegenerative disease in young adults and affects approximately 2 million people worldwide (Inglese, 2006). It is characterized by inflammation, demyelination in the central nervous system (CNS) and by axonal loss (Compston and Coles, 2008). MS affects both white and gray matter. Although cognitive impairment is encountered in approximately half of the MS population (Rao et al., 1991), the mechanisms leading to this cognitive impairment remain largely elusive.

A reduced white matter integrity in the whole MS brain has been shown (Ceccarelli et al., 2009; Cercignani et al., 2001; Rovaris and Filippi, 2007; Yu et al., 2008). The loss of integrity of white matter tracts has been suggested to be related to decreased brain synchrony (Arrondo et al., 2009) and impaired cognitive performance in MS using diffusion tensor imaging (DTI) (Dineen et al., 2009; Hulst et al., 2013; Shu et al., 2011). Reduced interhemispheric synchronization has been found in MS patients compared to healthy controls by Leocani et al. (2000) and by Cover et al. (2006).

* Corresponding author at: UZ Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium. Tel.: +32 2 477 64 10.
E-mail addresses: Jeroen.Van.Schependom@vub.ac.be (J. Van Schependom), Jeroen.Gielen@vub.ac.be (J. Gielen), Jorne.Laton@vub.ac.be (J. Laton), Marie.dhooghe@mscenter.be (M.B. D'hooghe), Jacques.DeKeyser@uzbrussel.be (J. De Keyser), Guy.Nagels@vub.ac.be (G. Nagels).

http://dx.doi.org/10.1016/j.nicl.2014.01.012

Although several structural MRI measures show significant correlation with cognitive functioning (Leocani et al., 2000) no sufficient explanation of cognitive impairment is available (Schoonheim et al., 2013). Recent research has focused on the construction of both structural and functional networks to understand the neural mechanisms that lead to cognitive impairment in MS.

Networks based on structural properties of the brain have already revealed alterations in network structure (Griffa et al., 2013). He et al. (2009) used structural MRI and networks based on cortical thickness measurements to demonstrate a small-world network efficiency loss proportional to the white matter lesion load and provided provisional evidence of MS as a disconnection disease. Applying DTI Shu et al. (2011) also showed global and local efficiency losses in MS patients compared to controls. However, in a study including both MRI and network fMRI measures, no MRI-parameters predicted cognition (Schoonheim et al., 2013).

Functional networks have also revealed network alterations. Leocani et al. have reported altered coherence in MS patients compared to healthy controls based on resting-state EEG, with more striking differences in cognitively impaired (CI) patients. They suggested corticocortical disconnection caused by demyelination and axonal loss to be responsible for the observed cognitive decline (Leocani et al., 2000). Partial functional disconnection in the temporal lobes was found to be associated to cognitive impairment by Hardmeier et al. (2012) who used MEG and synchronization likelihood to construct networks. On the same patient cohort, an increased normalized path length (lambda) and clustering coefficient (gamma) were found by Schoonheim et al. (2013) as indicators of a more regular topology.

As cognitive impairment in MS and its origins are not well understood we decided to construct networks based on a classic P300 paradigm experiment in which the patients are supposed to pay full attention. As attention is frequently impaired in MS (Rao et al., 1991), we expect correlations with cognitive functioning. To the best of our knowledge, this is the first study to assess networks based on the 'brain-in-action' in MS and to correlate these findings with an extensive neuropsychological battery.

Several difficulties exist when one tries to assess network-properties based on EEG data. Volume conduction and the influence of the reference electrodes give rise to the detection of multiple sources at one electrode. Several techniques have been devised to circumvent this problem. Nolte et al. (2004) have proposed the imaginary part of coherency as a measure independent of volume conduction and Stam et al. (2007) have proposed the Phase Lag Index (PLI) reasoning that a consistent phase lag cannot be explained by volume conduction. In total we have considered six methods to construct networks: correlation, correlation of the amplitudes in the frequency domain, coherence (alpha, beta, delta and theta), partial correlation, the imaginary part of coherency and the PLI. When assessing and constructing networks based on EEG data, we have to accept that every method has its disadvantages. The best way to go seems therefore, the combination of different network measures in order to ensure a complete description of the observed network. We also suppose that the problem of volume conduction and the influence of the reference electrodes are comparable for all patients. Therefore, we do not expect our results to be affected by these artifacts.

In this paper, we present the network differences in terms of edge weights, clustering coefficient, path length, modularity and degree between a cognitively preserved (CP) and a cognitively impaired (CI) group of MS patients for different methods that are frequently used to construct networks. As MS is considered a disconnection disease we expect significant differences from the network measures specifically designed not to be influenced by common sources (like the phase lag index and the imaginary part of the coherency). We hope that by investigating different methods to construct networks, we will be able to give a more robust interpretation of the networks.

2. Methods

2.1. Patient cohort

In the National MS Center Melsbroek (Belgium) patients regularly undergo neuropsychological testing to assess their cognitive performance. As part of the clinical assessment a neurophysiological assessment is included as well. MS is a disease in which several cognitive domains are deteriorated. The traditional measure used in neurophysiological studies in MS is the P300, a large positive wave following an unexpected stimulus and representing information processing speed and a patient's attentional skills (Whelan et al., 2010).

2.2. Neuropsychological tests

The neuropsychological test battery used is the Neuropsychological Screening Battery for MS (NSBMS) developed by Rao et al. (1991) and includes the Paced Auditory Serial Addition Test (PASAT) to test information processing speed, the Controlled Long Term Retrieval (CLTR) to test memory impairment, the Controlled Oral Word Association Test (COWAT) to test verbal fluency and the Spatial Recall test (SRT) to assess visuospatial memory. This test battery assesses the cognitive domains most frequently impaired in MS and has been extensively validated.

A patient is denoted as CI when he fails two or more tests included in the NSBMS. Failing one test is defined as not obtaining the 5th percentile of a normal population.

2.3. EEG preprocessing

Digital electroencephalography (EEG) recordings were carried out using a Brainlab Measure station (OSG, Belgium). Ag–AgCl bridge electrodes were placed on the scalp using the international 10/20 system. Signals were digitized in a Shwarzer headbox (OSG, Belgium) at 250 Hz. A 50-Hz notch filter was applied.

The offline analysis was performed using SPM8 (Litvak et al., 2011) and included filtering (highpass at 1 Hz, lowpass at 30 Hz), epoching (starting 200 ms before the stimulus and ending 800 ms after it), artifact detection (max. voltage 80 μV, max. peak to peak voltage 120 μV and flat segment detection), robust averaging with a subsequent lowpass filter (again at 30 Hz) and finally a baseline correction. Only target stimuli (39 out of 132) were included for the analyses. The following electrodes were used for the subsequent analyses: F_7, F_3, F_z, F_4, F_8, T_3, T_5, T_4, T_6, C_3, C_z, C_4, P_3, P_z, P_4, O_1 and O_2. These electrodes are considered the nodes of the networks.

2.4. Network construction

We constructed for each patient different networks using different connectivity measures. All networks considered in this paper are weighted networks, i.e. there always exists a link (an edge) between every pair of electrodes. The only difference between the different networks lies in the strength of these links.

1. Pearson correlation (corr)
   The most frequently used method to construct networks based on EEG/MEG data is the Pearson correlation. The strength of the correlation denotes the weight a certain edge is given.
2. Partial correlation (Partialcorr)
   Partial correlation is defined as the correlation between two time signals after regressing out all other time series.
3. Frequency-domain correlation (corrFreq)
   The concept of correlation can be easily extended to the correlation of the Fourier spectra of the respective signals. In this case, the covariation of the amplitudes at the different frequencies is taken as edge-weight.

4. Coherence (alpha, beta, delta and theta)
   Coherency is defined as a normalized cross-spectrum:

$$C_{ij}(f) = \frac{S_{ij}(f)}{\sqrt{S_{ii}(f)S_{jj}(f)}}$$

   with $S_{ij}(f)$ the cross-spectrum between signals i and j. The coherence is then defined as the absolute value of this quantity. Averaging over the different frequency bands results in α (8–10 Hz), β (13–30 Hz), δ (1–4 Hz) and θ (4–8 Hz) coherence values. Coherency is essentially a generalization of correlation to the frequency domain (Nunez et al., 1997).
   All the preceding measures (correlation, frequency-domain correlation and coherency) are prone to the problem of 'common sources'. One brain source can generate activity on several electrodes due to volume conduction. Furthermore (for EEG) one needs a reference electrode which can induce spurious correlation and coherency between two electrodes. A first way to circumvent these artifactual correlations is by estimating the source amplitudes. However, this inversion is not unique and, therefore assumptions have to be made.
5. Imaginary part of coherency (ImagCoh)
   Nolte et al. (2004) argue that the imaginary part of the coherency cannot be generated by volume conduction. Their main argument is that the imaginary part of the coherency assesses only time-lagged processes and is zero in the case of zero time-lag. As volume conduction is supposed to be instantaneous, the imaginary part of the coherency is insensitive to it and measures therefore true interaction.
6. Phase Lag Index (PLI)
   The disadvantage of using the imaginary part of the coherency is the fact that this measure depends on amplitude and phase of the signals. Therefore, the PLI was proposed by Stam et al. as a measure to detect consistent phase lags between two signals. They argue that when two signals show a consistent phase lag, this cannot be caused by volume conduction (Stam et al., 2007).

2.5. Network analysis

2.5.1. Edge-strength

The most obvious parameters of a network to be compared are the plain edge-strengths. As there are 136 independent statistical tests (one for each edge strength) we will apply a correction for the multiple comparison problem as outlined in the Statistics section. A common approach to evaluate network structure is by choosing a cutoff value above which all connections are assumed to be one and below which all connections are set to zero. This, however, introduces an arbitrary element in the calculations as this cutoff can be chosen as the one that fits best the underlying hypothesis. Therefore we prefer to work with weighted networks in which every connection (vertex) has a weight between 0 and 1.

2.5.2. Degree

The degree of a node i is defined as the number of neighbors that node i has. Recently there has been ample research showing that the brain is divided in nodes with large degree (the rich hubs) connecting high-clustered regions (Sporns, 2013). This architecture would allow the brain to process information efficiently and to pass information fast from one region to the other. As we are considering weighted networks, we defined the degree of a node as the sum of all the weighted connections that node has.

2.5.3. Mean path length

The path length was calculated using the definition given in Stam et al. (2009). In short, the edge-weights are inverted resulting in an adjacency matrix in which the highest values denote the worst connections. On this matrix, the shortest path is calculated between every possible pair of nodes. The average path length is then defined by Stam et al. as the harmonic mean of all path lengths.

2.5.4. Clustering coefficient

In unweighted networks the clustering coefficient of a node i is defined as the number of connections between all neighbors of node i divided by the total number of possible connections. We adhered to the definition of a weighted version of the clustering coefficient given by Stam et al. (2009).

2.5.5. Small-worldness

For calculating the small-worldness parameter one needs a normalized clustering coefficient and a normalized path length. The normalized clustering coefficient is defined as the ratio of the clustering coefficient to the mean over N randomly rewired networks of the mean (over all electrodes) clustering coefficient. The normalized path length (lambda) is obtained by calculating for N randomly rewired networks the mean path length and dividing the path length obtained in the original network by this mean. The small-world index (sigma) is then defined as the ratio between the mean normalized clustering coefficient and the mean normalized path length and is often assumed to reflect the efficiency at which information can be processed by a network. A small world network is defined as a network with a mean path length comparable to the mean path in a random network (lambda ≈ 1) but with a higher clustering coefficient (gamma > 1) (Griffa et al., 2013). For these results N was arbitrarily set to 50.

2.5.6. Modularity

The definition for modularity was identical to the definition used by de Haan et al. (2012). Instead of a simulated annealing approach, we have used fixed modules (frontal, central, parietal, temporal-left, temporal-right and occipital).

2.6. Statistics

2.6.1. Correction for age

As age significantly differed between the CP and CI groups, we used linear regression on all network parameters to extract the linear effect of age out of the networks. We also report the results of these correlations.

2.6.2. Non-Gaussian statistics

It is well known in the case of correlations that the distribution turns out to be non-Gaussian and therefore Gaussian statistics (like t-test's) are not valid. One way to cope with this problem is to apply a transformation (typically arctanh(x)) in order to construct a Gaussian distribution. Another way, which is the approach we followed, is the use of non-parametric statistics. Therefore, the p-values reported in this article are p-values from the Wilcoxon-rank test.

2.6.3. The multiple comparison problem

Comparing a huge number of parameters between two groups of subjects is likely to give some significant results. We used the False Discovery Rate (FDR) method in order to detect significant differences (Benjamini and Hochberg, 1995).

2.6.4. Cohen's d as effect size (ES)

A major problem of this study might have been the large sample size. We could include over 300 MS patients and are therefore prone to detect significant differences that are not clinically meaningful, i.e. we might have overpowered this study (Friston, 2012). Therefore we report Cohen's d as an effect size estimator, which ought to give an impression of the separability of the groups.

3. Results

3.1. Patient cohort

After matching the neurophysiological and the neuropsychological database, a total of 312 patients could be included with at least one EEG and one NP assessment within 30 days. After preprocessing, 4 EEG measurements showed too many artifacts to be included. From the remaining 308 patients, the largest part was denoted CP (180/308, 58.4%). Age differed significantly between the two groups ($p < E-6$) and was therefore included as a covariate in all subsequent analyses. Both groups showed a similar gender distribution, 78 men (43%) were included in the preserved group and 50 men (41%) in the impaired group. Approximately 80% of our patient cohort suffered Relapsing Onset MS. Disease duration was significantly different between both groups ($p = 0.04$) but was highly correlated with age. When age was included as a covariate, disease duration did not differ significantly between both groups. The Expanded Disability Status Scale (EDSS) (Kurtzke, 1983) did not differ significantly either (see Table 1).

3.2. Edge weights

In Fig. 1 we see the results of the differential network weights between CI and CP patients. Considering correlation both in time and frequency domain, we see extensive networks. Almost every connection is significantly different comparing the cognitively impaired and preserved groups (see Fig. 1.A–B). Assessing the same results for coherence, one sees some centro-parietal differences in the α-range (Fig. 1.C) and few differences in the β-range (Fig. 1.D). Most differences seem to take place in the δ (1–4 Hz, Fig. 1.E) and θ (4–8 Hz, Fig. 1.F) range. The PLI shows only one significantly different edge (P3-Cz, Fig. 1.G). No differences were found with the imaginary part of coherency as a measure or using partial correlations (figures not shown).

3.3. Clustering coefficient

Considering the clustering coefficient, the most significant differences between CI and CP patients can be found using mere correlations ($p < E-7$ and ES ≈ 0.65 at electrode T4), the correlations in the frequency domain ($p < E-7$ and ES ≈ 0.67 at electrode Fz) and the coherence in both δ ($p < E-7$ and ES ≈ 0.64 at electrode F8) and θ ($p < E-6$ and ES ≈ 0.6 again at T3) range. This finding is in concordance with the results in the previous section, i.e. we already knew that these networks showed the most significant differences.

Furthermore it is interesting to note that for all measures the clustering coefficient in the preserved group is higher than in the impaired group. There seems to be an extensive variation in location reflective of the measure used (see Table 2).

3.4. Degree, modularity and path length

In Table 3, we have listed the differences in degree, modularity and path length. Significant differences in path length are found in the networks constructed with correlation, correlation in the frequency domain, coherence in alpha, delta and theta domains and PLI. The same structure of significance can be seen when assessing "degree" as parameter. Modularity did not show significant differences.

3.5. Age

We have also correlated all network parameters with age. These results can be found in Table 4. It is interesting to note that although no differences in modularity were found between CI and CP patients, modularity did correlate significantly with age. None or less significant results are found for degree, path length and clustering coefficient (data not shown).

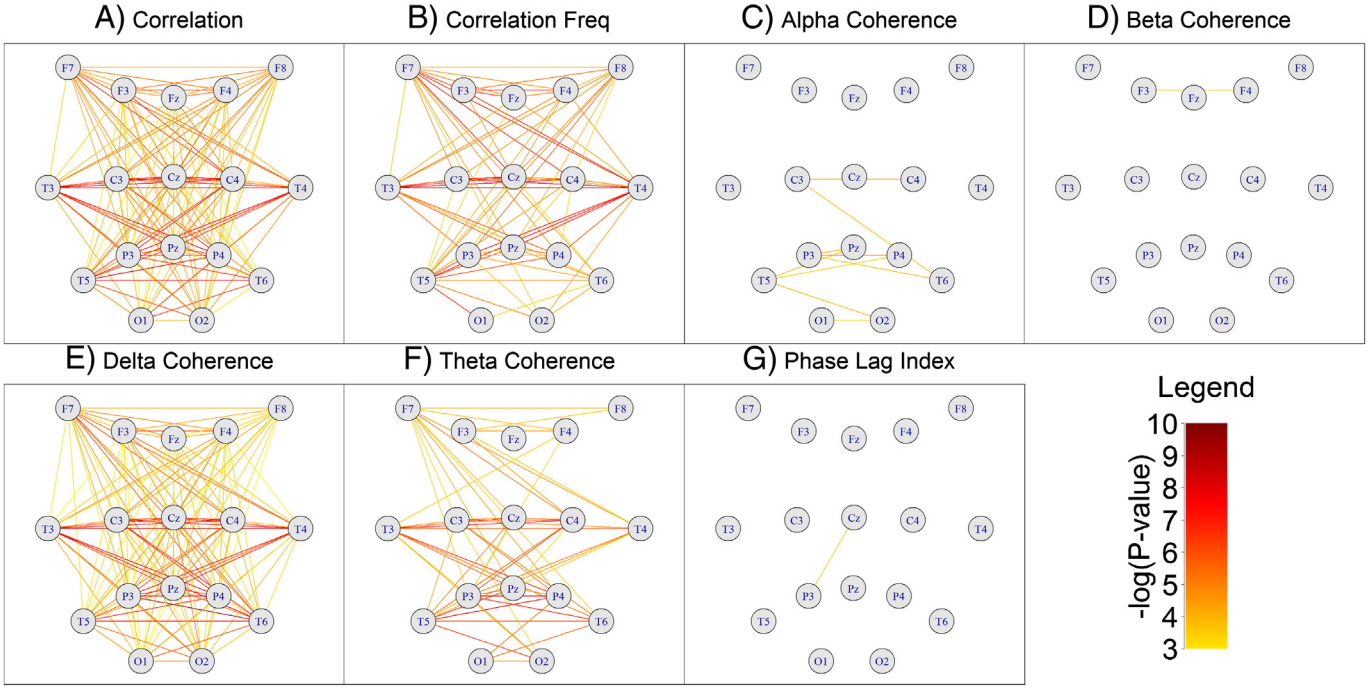

Fig. 1. Significantly different networks between the CP and the CI groups for different connectivity networks (A. Correlation, B. Correlation in the frequency domain, C. Coherence-alpha, D. Coherence-beta, E. Coherence-delta, F. Coherence-theta and G. PLI). Only edges that are significant using the FDR with a p-value of 0.05 are shown. Cohen's ds range from −0.4 to −0.75 indicating stronger connections for the preserved group for all measures.

Table 1
Patient characteristics.

	CI	CP	p-Value
N	128	180	–
Gender (% male)	35	43	–
MS-type (% RO)	77	81	–
Age (mean ± SD)	55 ± 12	49 ± 11	3E−6
Disease duration (mean ± SD)	19 ± 11	16 ± 10	0.04
EDSS-score	6.8	6.0	0.80

In this table, we provide the most important clinical features. MS-type is given as the percentage in each group that is affected by the Relapsing Onset type (RO) as opposed to the Progressive Onset type (PO).

3.6. Small-worldness

In Table 5, we observe significantly higher normalized path lengths (lambda) in the intact group in the networks constructed via correlation and coherence (beta and delta). For the same connectivity measures, the normalized clustering coefficients (gamma) are higher in the impaired group as is the small-worldness parameter sigma (= gamma/lambda).

In Fig. 2 we have plotted the statistical significance of the normalized clustering coefficients for all electrodes for the networks constructed with correlation, corrFreq and coherence (delta and theta). Correlation and coherence in the theta and delta range indicate that especially the clustering coefficients of the central and frontal areas are related to cognition whereas the correlation in the frequency domain returns more temporo-parietal clustering coefficients.

4. Discussion

In this paper we assessed EEG-recordings of 308 MS patients during an auditory oddball task. We have constructed networks using several methods (correlation, correlation in the frequency domain, coherency (alpha, beta, delta and theta), Phase Lag Index, partial correlation and the imaginary part of coherency) and calculated the most commonly reported network parameters (edge weights, clustering coefficient, mean path length, degree, modularity and small-worldness).

The main goal was to determine which method could return the most information on cognitive impairment in MS and whether the combination of different methods could yield additional and valuable information.

4.1. Edge weights

Using correlation in time and frequency domain as measures to construct the networks we observed a large network of significantly different network connections (edge weights). Differences that seem to be caused by differences in the delta and – in order of decreasing importance – theta, alpha and beta bands as pointed out by the coherence analysis. None or very few connections survived the FDR when the networks were constructed using partial correlation, PLI or the imaginary part of the coherency. Higher edge weights were consistently found in the CP group.

Finding higher correlations in the CP group might not be surprising as increased P300 amplitudes have been consistently found in the literature (Kiiski et al., 2011; Polich et al., 1992). Networks constructed using coherence highlight the importance of the delta (1–4 Hz) and theta (4–8 Hz) and to a lesser extent alpha (8–12 Hz) waves in the cognitive functioning in concordance with results from traditional EEG analyses (Kiiski et al., 2012). Furthermore, the most important correlations seem to be located at the temporo-parietal region, a region that has already emerged in a resting-state MEG study (Schoonheim et al., 2013). It can also be noted that almost all (98%) possible interhemispheric connections are related to cognition whereas only 60% of the intrahemispheric connections light up — using correlation as network measure (see Fig. 1.A). This supports recent evidence of the implication of the corpus callosum in the emergence of cognitive impairment in MS (Llufriu et al., 2012). The fact that we found higher correlations in the CP group seems to contradict Hawellek et al. (2011) who found an increased functional connectivity in MS patients based on BOLD signals and Schoonheim et al. (2013) who found increased synchronization in MS patients using resting state MEG. Hawellek et al., however, assessed MS patients in the early stages of the disease whereas we assessed a more general MS population. Therefore, we would like to argue that their results can be explained by compensation mechanisms. The alternative explanation offered by Hawellek et al., i.e. a loss of white matter tracts resulting in a reduced diversity in large-scale cortical dynamics, seems not to be supported by our results.

4.2. Clustering coefficient

The differences found using the clustering coefficient seem to correspond to the differences found in the edge weights. However, several clustering coefficients calculated using the PLI turned out to depend on a patient's cognitive status (the three most significant ones being T3, T6 and C3, $p < E-4$) while no significant differences were found when assessing the edge weights. This finding seems to indicate that, at least for the PLI, the clustering coefficient contains additional information or higher-quality information as it might reduce the levels of noise by multiplying several edge weights.

Previous studies have shown an increased normalized path length lambda and normalized clustering coefficient gamma comparing MS patients to healthy controls (Schoonheim et al., 2013) in agreement with our results in which CI patients show greater gamma and lambda. A decreased clustering coefficient and path length were recently shown when comparing Alzheimer patients with healthy controls (Stam et al., 2009).

Table 2
Three most important clustering coefficients.

	p-1	Es-1	L-1	p-2	Es-2	L-2	p-3	Es-3	L-3
Correlation	**7.566**	**0.657**	**T4**	**7.536**	**0.654**	**F8**	**7.421**	**0.646**	**Pz**
CorrFreq	**7.856**	**0.673**	**Fz**	**7.744**	**0.666**	**F3**	**7.692**	**0.657**	**F4**
AlphaCoh	**3.327**	**0.431**	**Fz**	**3.130**	**0.422**	**F3**	2.909	0.390	P4
BetaCoh	2.541	0.304	C4	2.342	0.297	F8	2.281	0.287	F7
DeltaCoh	**7.692**	**0.641**	**F8**	**7.598**	**0.636**	**Pz**	**7.508**	**0.647**	**T5**
ThetaCoh	**6.193**	**0.595**	**T3**	**6.011**	**0.592**	**Fz**	**6.005**	**0.590**	**P4**
PLI	**4.832**	**0.506**	**T3**	**4.580**	**0.483**	**T6**	**4.351**	**0.473**	**C3**
Partialcorr	1.176	−0.178	F8	1.000	0.207	Cz	0.6325	−0.125	T4
ImagCoh	1.801	−0.231	O2	1.462	−0.235	T5	1.243	−0.181	T6

In this table, we have shown the three electrodes at which – for each connectivity measure – the most significantly different clustering coefficients are observed. The p-values are given as $-\log10(p)$ in columns p-1, p-2 and p-3. Effect sizes in Es-1, Es-2 and Es-3, the locations in L-1, L-2 and L-3. A Bonferroni corrected p-value of 0.05, corresponds to $-\log10(0.05/17) = 2.53$. Effect sizes are given as 'preserved minus impaired'. Clustering coefficients that are both significant ($-\log10(p) > 2.53$) and have at least a moderate effect size (ES > 0.4) are shown in bold.

Table 3
Degree, modularity and path length.

	Degree			Modularity		Path length	
	P	ES	Pos	P	ES	P	ES
Correlation	**7.725**	**0.643**	**C4**	2.002	0.286	**7.434**	**−0.653**
CorrFreq	**8.409**	**0.719**	**T4**	0.805	0.118	**6.716**	**−0.630**
AlphaCoh	**3.357**	**0.427**	**P3**	0.138	0.036	2.698	−0.380
BetaCoh	2.075	0.283	F4	0.213	−0.048	1.677	−0.311
DeltaCoh	**7.902**	**0.607**	**Cz**	0.283	0.071	**7.418**	**−0.642**
ThetaCoh	**6.701**	**0.615**	**C4**	0.268	0.089	**6.184**	**−0.616**
PLI	**4.657**	**0.473**	**F7**	0.664	−0.114	**4.150**	**−0.426**
Partialcorr	1.120	0.131	F7	0.809	−0.155	0.069	0.027
ImagCoh	1.476	−0.222	F4	0.028	−0.047	1.155	0.199

The observed differences in several network parameters between the two groups. This first column denotes the observed p-values. p-Values are given as $-\log10(p)$, a p-value of 0.05 corresponds to 1.310. Considering modularity and path length, all values higher than 1.310 are therefore significant. The second column is for each measure Cohen's d as effect size given as 'preserved minus impaired'. For degree, the channel at which the most significantly different degree is found is given as well (Bonferroni corrected p-value corresponds to $-\log10(0.05/17) = 2.53$). All results exceeding significance and having at least a moderate effect size (abs(ES)>0.4) are shown in bold.

The significant clustering coefficients found in the networks based on the PLI seem to indicate that – in the case of the PLI – the clustering coefficient is more related to cognition than the edge weights. Given that the PLI reflects true changes in brain synchronization and is designed not to be influenced by volume conduction, we consider this as further evidence of an impaired synchronization leading to reduced cognitive functioning in MS.

4.3. Degree, modularity and path length

We see the same recurrent pattern when assessing the networks' degrees, modularity and mean path length. Again additional significance is reached in the PLI − network for degree (at electrode F7, $p < E-4$) and mean path length. No differences were found for modularity.

4.4. Small-worldness

We found that lambda showed significant differences for correlation and coherence in alpha and theta domains, which is in agreement with the results obtained by the mean path length. In contrast to our previous findings, lambda seems not to depend on cognitive status when assessed using PLI or the correlation in the frequency domain. Although we also found differences in the small-world parameter sigma, its absolute value in the impaired group (1.053 ± 0.033) and the preserved group (1.034 ± 0.030) does not allow us to consider the constructed networks as real small-world (Watts and Strogatz, 1998).

4.5. Age

Finally, we assessed the correlations with age and although the same significance pattern emerges, there are remarkable correlations with modularity. A higher age resulted in higher modularity. Modularity has, however, been suggested to degrade with age (de Haan et al., 2012; Meunier et al., 2009). We also observed almost all network weights decreasing with increasing age. We expect that the increased modularity at higher age is a consequence of the weaker edge weights and the fixed modules we have used instead of the simulated annealing approach.

4.6. Different network measures

Although we constructed different networks with diverse techniques, the choice of network measure does not seem to influence the final results. It stands without doubt that coherence returns the most information due to the selection of frequency ranges. When one has to limit oneself to one parameter, the PLI seems a viable candidate. The lack of significant results for both partial correlation and the imaginary part of the coherency may have different causes. It has been shown that the imaginary part of the coherency was less useful than coherence to assess experimental effects (Wheaton et al., 2005).

4.7. Cognitive impairment in MS as a disconnection symptom?

Network deficiencies have already been shown in MS. He et al. (2009) constructed networks based on cortical thickness and reported a network efficiency loss proportional to the white matter lesions. Shu et al. (2011) showed structural alterations in white matter networks between MS and healthy controls by applying DTI. Furthermore, damage to the corpus callosum has already been suggested to be associated with cognitive impairment (Llufriu et al., 2012). As the corpus callosum connects both cerebral hemispheres, we may interpret the observed importance of interhemispheric connections as a proxy of the importance of the corpus callosum white matter tracts.

Table 4
Correlations with age.

	Degree			Modularity		Path length	
	P	ES	Pos	P	ES	P	ES
Correlation	**4.547**	**−0.236**	**O2**	**6.429**	**0.285**	**2.761**	**0.178**
CorrFreq	**6.209**	**−0.280**	**P3**	**4.196**	**0.226**	**3.812**	**0.214**
AlphaCoh	0.436	−0.052	T6	0.474	0.055	0.108	0.016
BetaCoh	0.307	−0.039	F7	0.184	−0.026	0.025	0.004
DeltaCoh	**3.542**	**−0.205**	**T6**	**2.460**	**0.166**	**2.296**	**0.159**
ThetaCoh	**3.333**	**−0.198**	**T6**	**3.302**	**0.197**	**2.168**	**0.154**
PLI	**3.181**	**−0.193**	**Cz**	0.403	0.049	**1.509**	**0.123**
Partialcorr	1.564	−0.126	O2	0.411	−0.049	0.103	0.015
ImagCoh	1.773	0.136	T5	0.806	−0.081	0.815	−0.082

The observed correlations of the different network parameters with age. A higher degree and lower modularity are found in younger patients. We retain the same table-structure. p-Values are denoted as $-\log10(p)$ and all values above 1.31 are significant considering modularity and path length and above 2.53 considering degree. The second column of each parameter denotes the correlation coefficient. For degree we only report the degree of the channel which correlates best with age.

Table 5
Small-world parameters.

	Lambda				Gamma				Sigma			
	CI	CP	P	ES	CI	CP	P	ES	CI	CP	P	ES
Correlation	**1.02**	**1.03**	**5.37**	**−0.51**	**1.06**	**1.03**	**5.23**	**0.48**	**1.04**	**1.03**	**3.76**	**−0.38**
CorrFreq	1.00	1.01	0.99	−0.24	**1.01**	**1.00**	**4.36**	**0.31**	**1.01**	**1.00**	**4.43**	**−0.33**
AlphaCoh	1.08	1.13	0.97	−0.20	1.13	1.12	0.69	0.18	1.05	1.04	0.23	−0.08
BetaCoh	**1.06**	**1.10**	**1.57**	**0.21**	1.08	1.09	0.83	−0.15	1.02	1.02	0.13	0.01
DeltaCoh	**1.05**	**1.06**	**6.01**	**−0.56**	**1.10**	**1.05**	**6.19**	**0.59**	**1.05**	**1.03**	**5.11**	**−0.55**
ThetaCoh	**1.04**	**1.06**	**4.19**	**−0.45**	**1.09**	**1.05**	**4.51**	**0.47**	**1.04**	**1.04**	**3.27**	**−0.35**
PLI	**1.01**	**0.97**	**1.89**	**−0.22**	1.10	1.10	0.02	0.06	1.09	1.09	0.14	0.01
Partialcorr	1.04	1.03	0.30	0.07	1.05	1.05	0.04	0.01	1.01	1.01	0.32	−0.06
ImagCoh	0.998	1.08	1.02	−0.19	1.07	1.09	0.66	−0.15	1.08	1.10	1.08	0.18

Differences in normalized path length (lambda), normalized mean clustering coefficient (gamma) and the ratio between lambda and the mean clustering coefficient (sigma) known as the small-worldness parameter. For each variable we show the mean value in the impaired group (CI), preserved group (CP), −log10(p-value) and the effect size (ES). Significant results (-log10(p)>1.31) are shown in bold.

4.8. Limitations

Although we used the standard and extensive neuropsychological test battery (the NSBMS) the definition of cognitive impairment is not a perfectly objective measure of cognition and some noise is to be expected.

The correlations with age should be interpreted with caution as age is highly correlated with disease duration in this sample.

We considered calculating networks on averaged EEG data recorded when performing a task interesting as it shows the brain in action. However, we have to be careful with the interpretation as the connections reflect mean connections over the duration of the epoch and several consecutive processes are known to take place (stimuli perception, comparison of incoming stimulus with the stimuli in mind and counting).

We could only take into account 17 EEG electrodes, which seem a small number compared to recent MEG studies. However, it can be noted that in order to deal with the enormous amount of data in most MEG studies, one averages over the electrodes of certain areas. And although this might be the weakest point in our research, it might also be the strongest as it shows the applicability of advanced graph theoretical analysis methods on easily accessible data.

An important remark on all studies is the possible influence of differential signal-to-noise ratios (SNR) on the differences found in the constructed networks. Although we are aware that these SNR differences can heavily influence our (numerical) results, we expect a global influence. Therefore, we do not expect these SNR differences to explain the observed differential patterns.

4.9. Summary

In this study we constructed networks based on task-related averaged EEG data, collected in a clinical setting and linked these data to the MS patients' cognitive status. We have shown that all measures used to construct networks yield very similar results. The PLI, however, seems to be the best choice (for cognitive impairment in MS) when only one measure is to be used.

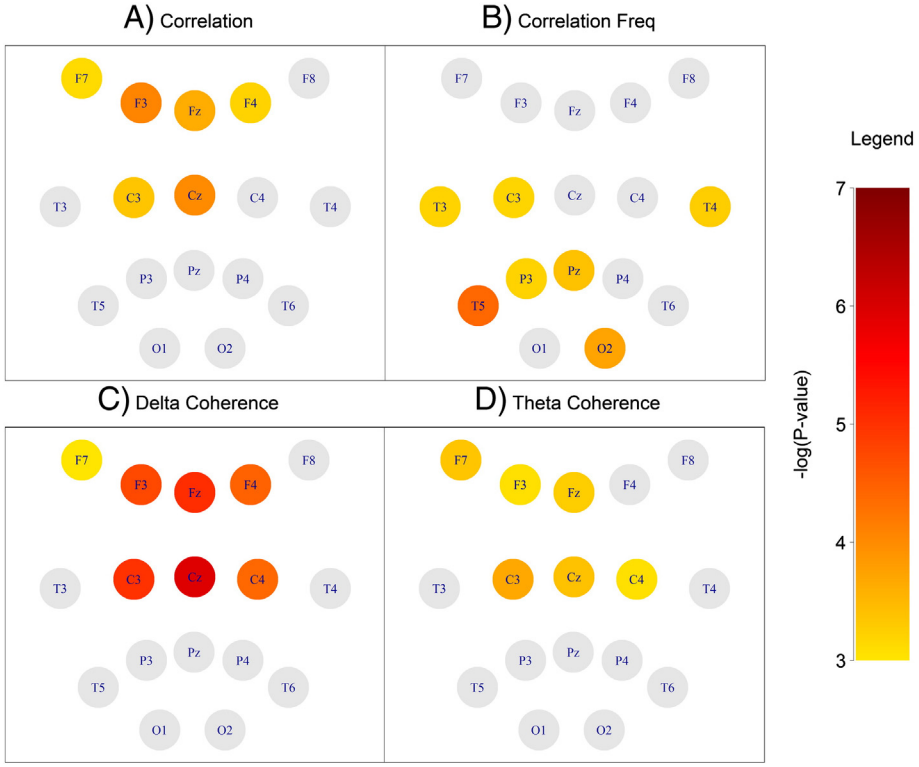

Fig. 2. Significantly different normalized clustering coefficients.

This study clearly shows the possibilities of the application of graph theoretical analysis methods to low-cost well-known data acquisition methods and may help to further enlighten the mechanisms leading to cognitive impairment in MS.

5. Conclusion

In summary, we can state that cognitive impairment in MS seems to stem from large-scale neural disconnection mechanisms, most probably involving the white matter tracts traveling through the corpus callosum. As we used low-cost and well-known data acquisition methods, these results may help to develop a standardized algorithm to detect cognitive impairment in MS.

Funding

JVS is a doctoral fellow of the Flanders Research Foundation (aspirant-FWO). GN holds the "Biogen Idec — National MS Center Melsbroek chair for neurophysiological research in multiple sclerosis" and the "Merck-Novartis joint research chair for neurophysiological pattern recognition in multiple sclerosis" at the Vrije Universiteit Brussel. The EDMUS clinical databasing effort in the National MS Center Melsbroek is supported by a grant from Teva Belgium.

Acknowledgments

We gratefully acknowledge the assistance of Mrs Ann Van Remoortel and the other MS-research nurses from the national MS center Melsbroek, who participated in this study.

References

Arrondo, G., Alegre, M., Sepulcre, J., Iriarte, J., Artieda, J., Villoslada, P., 2009. Abnormalities in brain synchronization are correlated with cognitive impairment in multiple sclerosis. Mult. Scler. 15, 509–516.

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: a practical and powerful approach to multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300.

Ceccarelli, A., Rocca, M. a, Valsasina, P., Rodegher, M., Pagani, E., Falini, A., Comi, G., Filippi, M., 2009. A multiparametric evaluation of regional brain damage in patients with primary progressive multiple sclerosis. Hum. Brain Mapp. 30, 3009–3019.

Cercignani, M., Inglese, M., Pagani, E., Comi, G., Filippi, M., 2001. Mean diffusivity and fractional anisotropy histograms of patients with multiple sclerosis. AJNR Am. J. Neuroradiol. 22, 952–958.

Compston, A., Coles, A., 2008. Seminar Multiple sclerosis. 372.

Cover, K.S., Vrenken, H., Geurts, J.J.G., van Oosten, B.W., Jelles, B., Polman, C.H., Stam, C.J., van Dijk, B.W., 2006. Multiple sclerosis patients show a highly significant decrease in alpha band interhemispheric synchronization measured using MEG. Neuroimage 29, 783–788.

De Haan, W., van der Flier, W.M., Koene, T., Smits, L.L., Scheltens, P., Stam, C.J., 2012. Disrupted modular brain dynamics reflect cognitive dysfunction in Alzheimer's disease. Neuroimage 59, 3085–3093.

Dineen, R.A., Vilisaar, J., Hlinka, J., Bradshaw, C.M., Morgan, P.S., Constantinescu, C.S., Auer, D.P., 2009. Disconnection as a mechanism for cognitive dysfunction in multiple sclerosis. Brain 132, 239–249.

Eguíluz, V.M., Chialvo, D.R., Cecchi, G.a., Baliki, M., Apkarian, a.V., 2005. Scale-free brain functional networks. Phys. Rev. Lett. 94, 018102.

Friston, K., 2012. Ten ironic rules for non-statistical reviewers. Neuroimage 61, 1300–1310.

Griffa, A., Baumann, P.S., Thiran, J., Hagmann, P., 2013. Structural connectomics in brain diseases. Neuroimage 80, 515–526.

Hardmeier, M., Schoonheim, M.M., Geurts, J.J.G., Hillebrand, A., Polman, C.H., Barkhof, F., Stam, C.J., 2012. Cognitive dysfunction in early multiple sclerosis: altered centrality derived from resting-state functional connectivity using magneto-encephalography. PLoS One 7, e42087.

Hawellek, D.J., Hipp, J.F., Lewis, C.M., Corbetta, M., Engel, A.K., 2011. Increased functional connectivity indicates the severity of cognitive impairment in multiple sclerosis. Proc. Natl. Acad. Sci. 19066–19071 (November).

He, Y., Dagher, A., Chen, Z., Charil, A., Zijdenbos, A., Worsley, K., Evans, A., 2009. Impaired small-world efficiency in structural cortical networks in multiple sclerosis associated with white matter lesion load. Brain 132, 3366–3379.

Hulst, H.E., Steenwijk, M.D., Versteeg, A., Pouwels, P.J.W., Vrenken, H., Uitdehaag, B.M.J., Polman, C.H., Geurts, J.J.G., Barkhof, F., 2013. Cognitive impairment in MS: impact of white matter integrity, gray matter volume, and lesions. Neurology 80, 1025–1032.

Inglese, M., 2006. Multiple sclerosis: new insights and trends. Am. J. Neuroradiol. 27, 954–957.

Kiiski, H., Reilly, R.B., Lonergan, R., Kelly, S., Brien, M.O., Kinsella, K., Bramham, J., Burke, T., Donnchadha, S.O., Nolan, H., Hutchinson, M., Tubridy, N., Whelan, R., 2011. Change in PASAT performance correlates with change in P3 ERP amplitude over a 12-month period in multiple sclerosis patients. J. Neurol. Sci. 305, 45–52.

Kiiski, H., Reilly, R.B., Lonergan, R., Kelly, S., O'Brien, M.C., Kinsella, K., Bramham, J., Burke, T., O Donnchadha, S., Nolan, H., Hutchinson, M., Tubridy, N., Whelan, R., 2012. Only low frequency event-related EEG activity is compromised in multiple sclerosis: insights from an independent component clustering analysis. PLoS One 7, e45536.

Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33, 1444–1452.

Leocani, L., Locatelli, T., Martinelli, V., Rovaris, M., Falautano, M., Filippi, M., Magnani, G., Comi, G., 2000. Electroencephalographic coherence analysis in multiple sclerosis: correlation with clinical, neuropsychological, and MRI findings. J. Neurol. Neurosurg. Psychiatry 69, 192–198.

Litvak, V., Kiebel, S., Phillips, C., Henson, R., Kilner, J., Barnes, G., Oostenveld, R., Daunizeau, J., Flandin, G., Penny, W., Friston, K., Umr, C., Neuroscience, L., Dynamics, B., Team, C., 2011. EEG and MEG data analysis in SPM8. Comput. Intell. Neurosci. 852961.

Llufriu, S., Blanco, Y., Martinez-Heras, E., Casanova-Molla, J., Gabilondo, I., Sepulveda, M., Falcon, C., Berenguer, J., Bargallo, N., Villoslada, P., Graus, F., Valls-Sole, J., Saiz, A., 2012. Influence of corpus callosum damage on cognition and physical disability in multiple sclerosis: a multimodal study. PLoS One 7, 1–7.

Meunier, D., Lambiotte, R., Fornito, A., Ersche, K.D., Bullmore, E.T., 2009. Hierarchical modularity in human brain functional networks. Front. Neuroinform. 3, 1–12.

Nolte, G., Bai, O., Wheaton, L., Mari, Z., Vorbach, S., Hallett, M., 2004. Identifying true brain interaction from EEG data using the imaginary part of coherency. Clin. Neurophysiol. 115, 2292–2307.

Nunez, P.L., Srinivasan, R., Westdorp, a F., Wijesinghe, R.S., Tucker, D.M., Silberstein, R.B., Cadusch, P.J., 1997. EEG coherency. I: statistics, reference electrode, volume conduction, Laplacians, cortical imaging, and interpretation at multiple scales. Electroencephalogr. Clin. Neurophysiol. 103, 499–515.

Polich, J., Romine, J.S., Sipe, J.C., Aung, M., Dalessio, D.J., 1992. P300 in MS a preliminary repor. Int. J. Psychophysiol. 155–163.

Rao, S.M., Gary, J.L., Bernardin, L., Unverzagt, F., 1991. Cognitive dysfunction in multiple sclerosis. I. Frequency, patterns, and prediction. Neurology 41, 685–691.

Rovaris, M., Filippi, M., 2007. Diffusion tensor MRI in multiple sclerosis. J. Neuroimaging 17 (Suppl. 1), 27S–30S.

Schoonheim, M.M., Geurts, J.J.G., Landi, D., Douw, L., van der Meer, M.L., Vrenken, H., Polman, C.H., Barkhof, F., Stam, C.J., 2013. Functional connectivity changes in multiple sclerosis patients: a graph analytical study of MEG resting state data. Hum. Brain Mapp. 34, 52–61.

Shu, N., Liu, Y., Li, K., Duan, Y., Wang, J., Yu, C., Dong, H., Ye, J., He, Y., 2011. Diffusion tensor tractography reveals disrupted topological efficiency in white matter structural networks in multiple sclerosis. Cereb. Cortex 21, 2565–2577.

Sporns, O., 2011. The human connectome: a complex network. Ann. N. Y. Acad. Sci. 1224, 109–125.

Sporns, O., 2013. Structure and function of complex brain networks. Dialogues Clin. Neurosci. 15, 247–262.

Sporns, O., Tononi, G., Kötter, R., 2005. The human connectome: a structural description of the human brain. PLoS Comput. Biol. 1, e42.

Stam, C.J., Nolte, G., Daffertshofer, A., 2007. Phase lag index: assessment of functional connectivity from multi channel EEG and MEG with diminished bias from common sources. Hum. Brain Mapp. 28, 1178–1193.

Stam, C.J., de Haan, W., Daffertshofer, A., Jones, B.F., Manshanden, I., van Cappellen van Walsum, a M., Montez, T., Verbunt, J.P. a, de Munck, J.C., van Dijk, B.W., Berendse, H.W., Scheltens, P., 2009. Graph theoretical analysis of magnetoencephalographic functional connectivity in Alzheimer's disease. Brain 132, 213–224.

Watts, D.J., Strogatz, S.H., 1998. Collective dynamics of "small-world" networks. Nature 393, 440–442.

Wheaton, L.A., Nolte, G., Bohlhalter, S., Fridman, E., Hallett, M., 2005. Synchronization of parietal and premotor areas during preparation and execution of praxis hand movements. Clin. Neurophysiol. 116, 1382–1390.

Whelan, R., Lonergan, R., Kiiski, H., Nolan, H., Kinsella, K., Bramham, J., Brien, M.O., Reilly, R.B.B., Hutchinson, M., Tubridy, N., O'Brien, M., 2010. A high-density ERP study reveals latency, amplitude, and topographical differences in multiple sclerosis patients versus controls. Clin. Neurophysiol. 121, 1420–1426.

Yu, C.S., Lin, F.C., Liu, Y., Duan, Y., Lei, H., Li, K.C., 2008. Histogram analysis of diffusion measures in clinically isolated syndromes and relapsing–remitting multiple sclerosis. Eur. J. Radiol. 68, 328–334.