Supplementary Information - Methods

Participants

Existing data of 83 MS patients and 34 healthy controls from the Amsterdam MS cohort were analyzed. These datasets were acquired as part of an ongoing clinical study at the Multiple Sclerosis Center Amsterdam, as described previously(1). Patients were diagnosed with clinically definitive MS according to the revised McDonald criteria(2) and involved relapsing-remitting MS (RRMS, N=59), secondary progressive MS (SPMS, N=16), and primary progressive MS (PPMS, N=8). All participants underwent clinical assessment consisting of history taking, neurological examination, blood tests, neuropsychological tests, structural MRI and MEG-recording. Educational level was determined using a Dutch classification system, ranking from 1 (did not finish primary education) to 7 (university degree) as described previously(3). Disability was classified using the Expanded Disability Status Scale (EDSS)(4).

Data acquisition

Neuropsychological evaluation

Directly after the MEG-sessions, neuropsychological tests were performed, consisting of the Brief Repeatable Battery of neuropsychological tests (BRB-N), expanded with the concept shifting test (CST), the Stroop test and the memory comparison test (MCT). This test-battery has been extensively validated in MS and evaluates executive functioning (CST), verbal memory (selective reminding test (SRT)), verbal fluency (word list generation), information processing speed (symbol digit modalities test (SDMT)), visuospatial memory (spatial recall test), working

memory (MCT) and attention (Stroop color and word test)(5). For each patient, a Z-score (corrected for age, gender and educational level) was calculated for each test, based on the mean and SDs of the complete healthy control group (HCs) from the original cohort. Z-scores were averaged for each domain separately and subsequently averaged into an average cognition Z-score, as previously described(6). Patients with a score lower than -2SD on at least two domains below the healthy control scores were considered ‘cognitively impaired’ (CI), while patients with a score below 1.5 to 2SD below HCs on at least two domains, while not fulfilling the CI criteria, were defined as ‘mildly cognitively impaired’ (MCI). Patients scoring better than MCI were considered ‘cognitively preserved’ (CP).

MRI-recordings

All subjects were scanned on a 3 Tesla whole-body magnetic resonance system (General Electric Signa-HDxt, Milwaukee, WI, USA), using an eight-channel phased-array head coil. The protocol included a three-dimensional T1-weighted fast spoiled gradient echo sequence for volumetric measurements (repetition time 7.8 ms, echo time 3 ms, inversion time 450 ms, flip angle 12 degrees, 1.0 mm sagittal slices, 0.9 x 0.9 mm2 in-plane resolution) and a threedimensional fluid attenuated inversion recovery sequence for white matter lesion segmentation (repetition time 8000 ms, echo time 125 ms, inversion time 2350 ms, 1.2 mm sagittal slices, 0.98 x 0.98 mm2 in-plane resolution)(7). Normalized gray matter volumes (NGMV), white matter volumes (NWMV), and whole brain volumes (NBV) were measured with SIENAX (FSL 5, FMRIB's Software Library, http://www.fmrib.ox.ac.uk/fsl(8), after lesion filling. Thalamic volumes were measured using FIRST (part of FSL), corrected for head size

with the V-scaling factor of SIENAX. All scans were inspected by an experienced rater (MMS).

MEG-recordings and pre-processing

MEG-data were acquired using a 306-channel whole-head MEG-system (Elekta Neuromag Oy, Helsinki, Finland), while participants were in supine position inside a magnetically shielded room (VacuumSchmelze GmbH, Hanua, Germany). A recording protocol of 3 minutes eyes-open and 5 minutes eyes-closed was followed, with a sample frequency of 1250 Hz. Only data from the eyes-closed session was used for further analysis. An antialiasing filter of 410 Hz and a high-pass filter of 0.1 Hz were applied online and other artifacts were removed offline using the temporal extension of Signal Space Separation (tSSS) in MaxFilter software with a sliding window of 10 s (Elekta Neuromag Oy, version 2.2.10)(9, 10). Before tSSS, malfunctioning channels were removed after careful visual inspection of the raw data [MF] (mean number of excluded channels was 6.3, range: 1–12). The participants' head position in relation to the MEG sensors was continuously recorded using signals from four head-localization coils(11). The head-localization coil positions were digitized, as well as the outline of the participants scalp (~500 points), using a 3D digitizer (Fastrak, Polhemus, Colchester, VT). Scalp surfaces of all subjects were coregistered to their structural MRIs using a surface-matching procedure, with an estimated resulting accuracy of 4 mm(12). A single best fitting sphere was fitted to the outline of the scalp as obtained from the coregistered MRI, which was used as a volume conductor model for the beamformer approach.

The automated anatomical labeling (AAL) atlas was used to define 78 cortical and 10 deep gray matter structures consisting of the amygdala, caudate, pallidum, putamen and thalamus. Broad-band (0.5-48 Hz) time-series were estimated for the centroid of each these ROIs by using a previously described atlas-based beamforming approach(13, 14). These time series were then used for further analysis: for each subject, 5 non-overlapping, artifact-free epochs of 16384 samples (13.1072 seconds) were selected [MF], based on careful visual inspection, and down sampled by a factor of 4. Inspection and further analysis was done with the in-house developed software package Brainwave (version 0.9.152.12.5), available from http://home.kpn.nl/stam7883/brainwave.html.

Time-series analyses

The time series were digitally filtered using a discrete Fast Fourier transform, to calculate the relative power for each of 6 classical EEG/MEG frequency bands (delta (0.5-4 Hz), theta (4-8 hz), alpha1 (8-10 Hz), alpha2 (10-13 Hz), beta (13-30 Hz), gamma 13-48 Hz)), and peak frequency, in each cortical ROI (n=78) and deep gray matter ROI (n=10) resulting in 6 sets of 5 epochs for all ROIs. Peak frequency and relative powers were estimated for each cortical and subcortical ROI separately. Additionally, cortical relative power was computed as mean relative power over all 78 cortical ROIs and epochs. The same was done for peak frequency to get the average cortical peak frequency. Deep gray matter relative powers and peak frequency were estimated as means over 10 deep gray matter ROIs (see also **table 1)** .

Statistical analysis

Baseline characteristics

Statistical analyses were performed in IBM SPSS Statistics 22.0 or Matlab (The Mathworks, version 7.14.0.739). Normality was checked by visual inspection and the Kolmogorov-Smirnov test. Univariate and multivariate linear models were used (or in the absence of normality non-parametric testing) to identify group differences, using age, gender and educational level as covariates.

If significant whole brain (i.e. cortical and subcortical) relative power differences were found within specific frequency bands, regional relative power values were compared between groups using a Mann-Whitney test. In order to determine which frequency bands correlated most strongly with overall cognition and each cognitive domain, a linear regression model was constructed, correcting for age, gender and education, to obtain coefficients for the correlation between average cognition Z-scores and each of the MEG variables (i.e. whole brain relative power in a frequency band, or peak-frequency). A p-value of <0.05 was considered statistically significant with correction for multiple comparisons using the false discovery rate (FDR), correcting for six frequency bands plus peak-frequency times eight cognitive domains(15). Furthermore, differences in whole brain relative powers and peak-frequencies between MS subtypes (regardless of cognition scores) were explored using a logistic regression model correcting for age, gender and education.

References

1. Tewarie P, Steenwijk MD, Tijms BM, Daams M, Balk LJ, Stam CJ, et al. Disruption of structural and functional networks in long-standing multiple sclerosis. Hum Brain Mapp. 2014;35(12):5946-61.
2. Polman CH, Reingold SC, Banwell B, Clanet M, Cohen JA, Filippi M, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol. 2011;69(2):292-302.
3. Tewarie P, Schoonheim MM, Stam CJ, van der Meer ML, van Dijk BW, Barkhof F, et al. Cognitive and clinical dysfunction, altered MEG resting-state networks and thalamic atrophy in multiple sclerosis. PLoS One. 2013;8(7):e69318.
4. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33(11):1444-52.
5. Eijlers AJ, Meijer KA, Wassenaar TM, Steenwijk MD, Uitdehaag BM, Barkhof F, et al. Increased default-mode network centrality in cognitively impaired multiple sclerosis patients. Neurology. 2017;88(10):952-60.
6. Amato MP, Portaccio E, Goretti B, Zipoli V, Ricchiuti L, De Caro MF, et al. The Rao's Brief Repeatable Battery and Stroop Test: normative values with age, education and gender corrections in an Italian population. Mult Scler. 2006;12(6):787-93.
7. Eijlers AJC, Meijer KA, van Geest Q, Geurts JJG, Schoonheim MM. Determinants of Cognitive Impairment in Patients with Multiple Sclerosis with and without Atrophy. Radiology. 2018:172808.
8. Smith SM, Zhang Y, Jenkinson M, Chen J, Matthews PM, Federico A, et al. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. Neuroimage. 2002;17(1):479-89.
9. Taulu S, Hari R. Removal of magnetoencephalographic artifacts with temporal signal-space separation: demonstration with single-trial auditory-evoked responses. Hum Brain Mapp. 2009;30(5):1524-34.
10. Taulu S, Simola J, Kajola M. MEG recordings of DC fields using the signal space separation method (SSS). Neurol Clin Neurophysiol. 2004;2004:35.
11. Fraschini M, Demuru M, Crobe A, Marrosu F, Stam CJ, Hillebrand A. The effect of epoch length on estimated EEG functional connectivity and brain network organisation. J Neural Eng. 2016;13(3):036015.
12. Whalen C, Maclin EL, Fabiani M, Gratton G. Validation of a method for coregistering scalp recording locations with 3D structural MR images. Hum Brain Mapp. 2008;29(11):1288-301.
13. Hillebrand A, Nissen IA, Ris-Hilgersom I, Sijsma NC, Ronner HE, van Dijk BW, et al. Detecting epileptiform activity from deeper brain regions in spatially filtered MEG data. Clin Neurophysiol. 2016;127(8):2766-9.
14. Hillebrand A, Barnes GR, Bosboom JL, Berendse HW, Stam CJ. Frequency-dependent functional connectivity within resting-state networks: an atlas-based MEG beamformer solution. Neuroimage. 2012;59(4):3909-21.
15. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society Series B-Methodological. 1995;57(1):289-300.