Supplementary Material

Supplementary Methods

MRI acquisition. Using 3.0 Tesla scanners (IRCCS San Raffaele: Philips Ingenia CX; University of Genoa and University of Alabama: Siemens Prisma; Kessler Foundation: Siemens Skyra) and standardized procedures for subjects positioning, the following brain MRI sequences were acquired from all subjects during a single session: a) axial T2*-weighted single-shot EPI for resting state (RS) functional (f) MRI (all scanners: TR=1560 ms; TE=35 ms, flip angle=70°; multi-band factor=2, matrix size=96×96; FOV=240 x 240 mm^2; 48 contiguous axial slices, 3 mm thick, number of volumes=320); b) variable flip angle 3D T2-weighted fluid-attenuated inversion recovery (FLAIR) turbo spin echo (Philips scanner: repetition time [TR]=4800 ms; echo time [TE]=270 ms; inversion time [TI]=1650 ms; matrix size=256 × 256; field of view [FOV]=256 × 256 mm^2; echo train length [ETL]=167; 192 contiguous sagittal slices, 1 mm thick; Siemens scanners: TR=5000 ms; TE=395 ms; TI=1800 ms; matrix size=256 × 256; FOV=256 × 256 mm^2; ETL=284; 192 contiguous sagittal slices, 1.05 mm thick), c) sagittal 3D T1-weighted sequence: (Philips scanner: TR=7 ms; TE=3.2 ms; TI=1000 ms; flip angle=8°; matrix size=256 × 256; FOV=256 × 256 mm^2; 204 contiguous sagittal slices, 1 mm thick; Siemens scanners: TR=2300 ms; TE=2.98 ms; TI=900 ms; flip angle=9°; matrix size=256 × 256; FOV=256 × 256 mm^2; 204 contiguous sagittal slices, 1 mm thick); and d) axial pulsed-gradient spin echo single shot diffusion-weighted echo planar imaging (EPI) (all scanners: 3 shells at b-value=700/1000/2855 s/mm^2 along 6/30/60 non-collinear directions and 10 b=0 volumes were acquired, FOV=240×233 mm, pixel size=2.14×2.69 mm, 56 slices, 2.3 mm-thick, matrix=112×85, TR=about 6000 ms, TE=about 80 ms and three additional b=0 volumes with reversed polarity of gradients for distortion correction). Acquisition for RS fMRI scans required about 8 minutes. During RS fMRI acquisition, subjects were asked to keep their eyes closed, to remain motionless and not to think of anything in particular. All subjects stated that they had not fallen asleep during scanning, according to a

questionnaire delivered immediately after the MRI session. The total duration of MRI acquisition was approximately 50 minutes.

Voxel-wise atrophy analysis. Voxel-based morphometry (VBM), as implemented in SPM12 (www.fil.ion.ucl.ac.uk/spm), was used to map modifications in regional gray matter (GM) volumes between multiple sclerosis (MS) patients and healthy controls (HC). The lesion-filled 3D T1-weighted images were used for a group-wise alignment: first, the images were segmented into different tissue types via the Segmentation routine in SPM12. Then, GM and white matter (WM) segmented images of all study participants, in the closest possible rigid-body alignment with each other, were used to produce GM and WM templates and to drive the deformation to the templates. At each iteration, the deformations, calculated using the Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) registration method [1], were applied to GM and WM, with an increasingly good alignment of study participant's morphology, to produce templates. Finally, an affine transformation that maps from the population average (DARTEL Template space) to Montreal Neurological Institute (MNI) space was calculated. GM and WM maps were spatially normalized, modulated for the Jacobian of the non-linear transformation and smoothed with an 8 mm Gaussian kernel. To define the DLPFC, Brodmann areas (BA) 9 and 46 were selected by BAs template (in which the DLPFCs are contained). For each study participant, the regional GM volume in the native space was obtained by summing values of the aforementioned maps within the mask.

DT MRI pre-processing. Preprocessing of diffusion-weighted imaging data included correction for off-resonance and eddy current induced distortions, as well as for slice-to-volume and subject movements, and signal dropout, using the Eddy tool within the FSL library (FSL version 6.0.1, www.fmrib.ox.ac.uk) [2].

The diffusion tensor (DT) was estimated in each voxel using the shell at b=700 and 1000 by linear regression [3] using the FMRIB's Diffusion Toolbox (FDT tool, FSL 5.0.5).

Construction of WM Atlas. To generate WM fiber bundles, a separate cohort of 44 HC (24 females, age 32 ± 12, range 18-55 years) underwent the same diffusion-weighted and 3D T1-

weighted MRI protocol described above, on the same 3.0 Tesla Philips Ingenia CX scanner used for the study at IRCCS San Raffaele.

Fiber orientation distribution (FOD) functions were computed using Multi-Shell, 3-Tissue Constrained Spherical Deconvolution, with group averaged response functions for WM, GM, and CSF using MRtrix3 software (www.mrtrix.org) [4]. FOD images of HC were averaged to create a study specific unbiased FOD population template. Spatial correspondence with the population template was achieved with an iterative registration and averaging approach [5]. Each subject's FOD and FA images were then registered to the template via a FOD-guided non-linear registration [6]. Probabilistic tractography[7] was then run using the FOD template to reconstruct the following WM tracts, which have been implicated in fatigue,[8-10] using a region of interest (ROI) approach and seed from the GM-WM interface: transcallosal fibers between left and right DLPFC, connections between ipsilateral caudate nucleus and thalamus, connections between ipsilateral caudate nucleus and DLPFC and connections between ipsilateral thalamus and DLPFC. For each WM tract, this approach is based on manual delineation of a "seed" ROI, based on anatomical landmarks (combining information provided by FA, FOD and T1-weighted images). Starting from the seed ROI, tractography reconstructs a probability map of the WM tract. An "end" ROI, namely another anatomical landmark to which the tract is known to connect the seed ROI was also used. Further "exclusion" ROIs were used to avoid the selection of undesired fibers and to optimize the selection of the tract of interest.

In details, the following seed and end ROIs were used for each WM tract:

- Transcallosal fibers between left DLPFC-right DLPFC: the spherical ROIs (10mm diameter) of DLPFC were shaped onto the BAs 46 and 9; the center of the left one: X:-42; Y:32; Z:30; and the right one: X: 42; Y:32; Z:30, in line with what suggested in a previous publication;[11]

- WM tracts connecting ipsilateral caudate nucleus-thalamus (left and right sides): the ROIs of these nuclei were the masks derived from FSL FIRST tool;

- WM tracts connecting ipsilateral caudate nucleus-DLPFC (left and right sides): the ROIs of caudate nuclei were derived from FSL FIRST tool, whereas the spherical seed ROIs (10mm diameter) of DLPFCs were shaped onto the BAs 46 and 9, as described above;

- WM tracts connecting ipsilateral thalamus-DLPFC (left and right sides): the ROIs of thalami were derived from FSL FIRST tool, whereas the spherical seed ROIs (10mm diameter) of DLPFCs were shaped onto the BAs 46 and 9, as described above.

For the subsequent analyses, mean FA and MD values of the aforementioned WM tracts were extracted by applying each atlas section as a mask on DWI of every single study participant.

RS fMRI pre-processing. RS fMRI data were pre-processed using the CONN toolbox (https://web.conn-toolbox.org/).[12] RS fMRI images were realigned to the mean of each session with a six-degree rigid-body transformation to correct for minor head movements. After rigid registration of realigned images to the lesion filled 3D T1-weighted scan, RS fMRI images were normalized to the MNI template using a standard affine transformation followed by non-linear warping. After detection of outliers (using the ART toolbox), images were smoothed with a 6 mm3 Gaussian filter. The five principal components derived from WM and CSF estimated with the anatomical component-based noise correction method (aCompCor),[13] and motion parameters with their first temporal derivatives were regressed out from RS fMRI time series as nuisance covariates. Outliers detected by the ART toolbox (if any) and spurious effects from the first two timepoints (to maximize magnetic equilibrium) were also regressed out from data. Finally, images were linearly detrended and band-pass filtered (0.01-0.1 Hz).

Supplementary References

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