**e-Methods**

Subjects. Subjects recruited in the current study are part of a prospective cohort followed at Hospital San Raffaele from 2008 to 2019. Inclusion criteria were: 1) right handedness; 2) no significant medical illnesses or substance abuse; 3) no other major systemic, psychiatric or neurological diseases; and 4) no contraindications to MRI (claustrophobia, pregnancy or breastfeeding). MS patients had also to be relapse- and steroid-free for at least three months before MRI acquisition and have a stable disease-modifying treatment from at least 6 months.

MRI acquisition. At baseline, the following MRI scans were collected from patients and HC using a 3.0 Tesla Philips Intera scanner (Philips Medical Systems, Eindhoven, The Netherlands): 1) T2\*-weighted single-shot echo planar imaging (EPI) sequence for RS functional MRI (fMRI) (repetition time [TR]=3000 ms/echo time [TE]=35 ms, flip angle=90°, field-of-view=240 mm2; matrix=128x128, slice thickness=4 mm, in-plane resolution=1.875x1.875 mm2, 200 sets of 30 contiguous axial slices); 2) 3D T1-weighted fast field echo (TR/TE=25/4.6 ms, flip angle=30°, field-of-view=230 mm2, matrix=256x256, in-plane resolution=0.89x0.89 mm2, slice thickness=0.8 mm, 220 contiguous axial slices); and 3) dual-echo (DE) turbo spin echo (TR/TE=2599/16-80 ms; flip angle=90; field-of-view=240mm2; matrix=256×256; in-plane resolution=0.93x0.93 mm2, echo train length=6; slice thickness=3 mm, 44 contiguous axial slices). For each scan, slices were positioned to run parallel to a line that joins the most infero-anterior and infero-posterior margins of the corpus callosum. Acquisition for RS fMRI scans required about 10 minutes. During acquisition, subjects were asked to keep their eyes closed, to remain motionless and not to think to anything in particular. All subjects stated that they had not fallen asleep during scanning, according to a questionnaire delivered immediately after the MRI session.

Conventional MRI analysis. Brain T2-hyperintense and T1-hypointense lesion volumes (LV) were quantified on DE scans and on 3D T1-weighted images using the Jim software (version 7, Xinapse Systems, Colchester, UK). After refilling of T1-hypointense lesions, baseline normalized brain volume (NBV), normalized grey matter (NGMV) and white matter (NWMV) volumes were calculated using FSL SIENAx. Automatic segmentation of the thalamus, putamen, pallidum, caudate, hippocampus, amygdala and accumbens was performed on 3D T1-weighted scans using the FSL FIRST software. Volume of these structures was multiplied by the head normalization factor derived from SIENAx and used to obtain the normalized volume of deep GM nuclei (NDGMV, i.e., the sum of thalamus, putamen, pallidum, caudate, amygdala and accumbens).

RS fMRI pre-processing. The main pre-processing steps were performed using SPM12 and REST software (http://resting-fmri.sourceforge.net/). After discarding the first two timepoints, RS fMRI scans were rigid-body realigned to the mean of each session. After rigid registration to the lesion filled 3D T1-weighted scan, RS fMRI images were non-linearly normalized to the Montreal Neurological Institute (MNI) template. Linear detrending and band-pass filtering (0.01-0.08 Hz) were performed to partially remove low-frequency drifts and physiological high-frequency noise. Finally, smoothing was performed using a 3D 6-mm isotropic Gaussian kernel.

Assessment of RS functional connectivity (FC) among networks. The temporal association among independent components (IC) estimated by the GIFT software and selected as networks of interest was explored using the functional network connectivity (FNC) toolbox (<http://mialab.mrn.org>). This was achieved by computing a constrained maximal lagged correlation between IC time courses, as previously described.1 The maximum possible lag between time courses was set at 6 seconds. In other words, we examined the correlation between two IC time courses, A and B, when B is circularly shifted from −6 to +6 seconds around A. The maximal correlation value and the corresponding lag were saved for each time course pairs.

Structural GM network analysis: pre-processing of 3D T1-weighted scans. Lesion-filled 3D T1-weighted scans were segmented into GM, white matter (WM) and cerebro-spinal fluid using SPM12, and non-linearly normalized using the Diffeomorphic Anatomical Registration using Exponentiated Lie Algebra2 registration method. Finally, GM maps were modulated and smoothed (3D 8-mm isotropic Gaussian kernel).

References

1. Jafri MJ, Pearlson GD, Stevens M, Calhoun VD. A method for functional network connectivity among spatially independent resting-state components in schizophrenia. NeuroImage 2008;39:1666-1681.

2. Ashburner J. A fast diffeomorphic image registration algorithm. NeuroImage 2007;38:95-113.