# ABNORMALITIES OF THE MAIN CORTICAL AND SUBCORTICAL FUNCTIONAL NETWORKS IN MS PATIENTS

**M.A. Rocca[1,2], P. Valsasina[1], L. Vacchi[1], V. Leavitt[3], G. Comi[2], A. Falini[4], M. Filippi[1,2]**

[1]Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, [2]Department of Neurology; [4]Department of Neuroradiology; San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy; [3]Department of Neurology, Multiple Sclerosis Clinical Care and Research Center, Columbia University Medical Center, New York, USA.

## INTRODUCTION and PURPOSE

• Resting-state (RS) functional MRI allows mapping function of large-scale neuronal networks of the human brain without any influence of between-subject differences related to task performance [1]. This is a critical issue in patients with multiple sclerosis (MS), who are characterized by heterogeneous disease manifestations and highly variable degrees of physical disability and cognitive impairment.

• RS fMRI studies performed so far in MS suggest that prominent RS functional connectivity (FC) changes occur in many RS networks and correlate with clinical and/or structural MRI measures. However, results of RS FC studies in MS are still controversial, thus making difficult a complete and univocal characterization of RS FC abnormalities in this condition [2, 3].

• Previous RS FC studies in healthy individuals [4] have identified seven major functional "hubs", each of which is associated with a functional brain network: there are four cortical networks (default mode, dorsal attention, visual and sensorimotor) and three subcortical networks (associated with thalamus, amygdala and cerebellum).

**Objectives:**

• To explore abnormalities of RS FC within the main cortical/subcortical brain networks in a large cohort of MS patients with different disease clinical phenotypes;

• To investigate the correlations between RS FC abnormalities and clinical status and cognitive performances.

## METHODS

**Subjects:** 215 right-handed MS patients (82/133 men/women, mean age=41.0 years, range=18.9-67.9 years) and 98 matched healthy controls (41/57 men/women, mean age=42.7 years, range=20.6-69.0 years) were recruited.

**Table 1**: main demographic and clinical characteristics of the enrolled study subjects.

| Table 1 | Healthy controls (N=98) | CIS patients (N=13) | RRMS patients (N=119) | SPMS patients (N=41) | BMS patients (N=29) | PPMS patients (N=13) | p* |
|---|---|---|---|---|---|---|---|
| Mean age (range) [y] | 42.7 (20.6-69) | 31.0 (19.9-43.0) | 37.5 (18.9-60.9) | 48.4 (26.0-66.1) | 44.7 (27.1-66.4) | 52.2 (42.2-67.9) | <0.001 |
| Men/women | 41/57 | 1/12 | 47/72 | 17/24 | 10/19 | 7/6 | 0.2§ |
| Median EDSS [range] | - | 1.5 (0.0-2.5) | 1.5 (0.0-4.5) | 6.0 (4.0-8.5) | 1.5 (1.0-3.0) | 6.0 (3.5-7.0) | 0.04§ |
| Mean disease duration (range) [y] | - | 0.4 (0.1-2.3) | 8.6 (0.1-24.0) | 18.9 (2.6-44.7) | 19.9 (15.7-26.0) | 15.6 (5.0-24.0) | 0.13 |

*ANOVA model; §Kruskall and Wallis test.
Abbreviations: RR=relapsing-remitting; MS=multiple sclerosis; SP=secondary progressive; BMS=benign multiple sclerosis; PP=primary progressive; SD=standard deviation; EDSS=Expanded Disability Status Scale.

**Study design:**

Clinical examination: rating of the EDSS score [5];

Neuropsychological evaluation: Brief Repeatable Battery of Neuropsychological Tests (BRB-N) [6]. Patients with at least two abnormal tests (≤2 SD below Italian normative values) were considered as cognitively impaired (CI) [7]. Z scores of verbal memory, visuo-spatial memory, attention and verbal fluency, as well as a global Z score of cognitive function [8] were obtained.

**MRI acquisition:**

3.0 T Philips Intera scanner:

• *RS functional MRI*: T2*-weighted EPI scans (TR/TE=3000/35 ms, matrix=128x128, FOV=240x240 $mm^2$, 30 axial slices with thickness=4 mm; 200 sets of images acquired while subjects lied still in the scanner);

• *Structural MRI*: dual-echo (DE) turbo spin-echo (TSE) for the assessment of T2 lesion volume (LV) and 3D T1-weighted scan for the quantification of normalized brain volume (NBV).

**RS fMRI analysis:**

• *RS fMRI pre-processing (AFNI and FSL software):*

- Realignment, co-registration to 3D T1-weighted scans, warping into MNI standard space and smoothing (8 $mm^3$ FWHM Gaussian filter);
- Rescaling of images to mean intensity, band-pass filtering and removal of nuisance regressors.

• *Assessment of RS FC:*

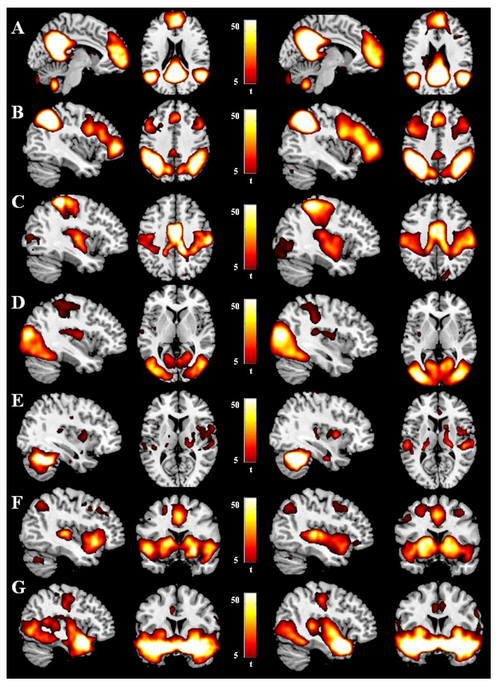
- Seed-based approach with the following seeds (spherical volumes having radius=6 mm) [4]: right posterior cingulate cortex (MNI coordinates: 4 -52 29); left inferior parietal cortex (MNI coordinates: -38 -53 39); right postcentral gyrus (MNI coordinates: 20 -44 57); left cuneus (MNI coordinates: -24 -80 18); left cerebellum, anterior lobe (MNI coordinates: -9 -56 -20); left thalamus, medial dorsal nucleus (MNI coordinates: -12 -19 8); right amygdala (MNI coordinates: 24 -6 -15).
- Correlation calculated between mean time series of the voxels within the seed and any other voxel of the brain; Fisher r-to-z transform used to improve gaussianity of the obtained correlation maps.

**Statistical analysis:**

- Between-group comparison of clinical and structural MRI variables: two-sample t test and ANOVA models for continuous variables, Mann-Withney and Kruskall Wallis test for categorical variables;
- SPM8: between-group voxelwise comparison of RS FC (two sample t test, age and sex adjusted);
- Effect of phenotype and cognitive impairment assessed on global and regional Z scores from each significant SPM cluster with ANOVA models, adjusted for age. Between-group comparisons decided *a priori,* on the basis of the clinical evolution of the disease: 1) HC *vs* CI *vs* CP; 2) HC *vs* CIS, HC *vs* PPMS, CIS *vs* RRMS, RRMS *vs* SPMS, RRMS *vs* BMS, SPMS *vs* BMS, CIS *vs* PPMS, SPMS *vs* PPMS and *vice versa*.
- Correlations of Z scores of RS FC with cognitive/structural MRI variables: linear regression models adjusted for age; correlations with EDSS and its functional scores: Spearman's rank correlation coefficient.

## RESULTS

**Figure 1**: spatial maps of RS FC with the selected seed regions (left=healthy controls; right=MS patients, p<0.05, FWE corrected).




**A** Seed region: right posterior cingulate cortex -> Default mode network (DMN).

**B** Seed region: left inferior parietal cortex -> Dorsal attention network (DAN).

**C** Seed region: right postcentral gyrus -> Sensorimotor RS FC network.

**D** Seed region: left cuneus -> Visual RS FC network.

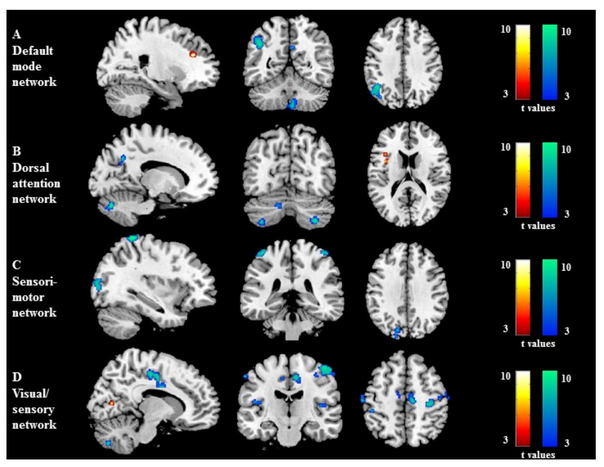
**E** Seed region: left anterior lobe of the cerebellum -> Cerebellar RS FC network.

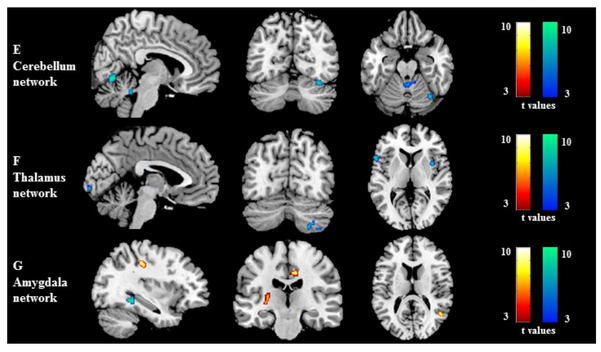
**F** Seed region: left thalamus, medial dorsal nucleus -> Thalamic RS FC network.

**G** Seed region: right amygdala -> Amygdala RS FC network.

Compared to HC, MS patients had decreased average RS FC in the DMN (p=0.002).

**Figure 2**: significant RS FC abnormalities in MS patients *vs* healthy controls in the seven functional networks examined (Blue-lightblue=clusters of decreased RS FC; Red-yellow=clusters of increased RS FC in MS patients *vs* controls).




Effects of clinical phenotype.

-Sensorimotor network RS FC decrease more significant in PPMS *vs* HC (p=0.04) and in SPMS *vs* RRMS (p=0.03).

-**CIS patients:** lower RS FC in frontal regions of the DMN (p=0.004 and 0.01), left precuneus of the DAN (p=0.05), and parietal, occipital and cerebellar regions of the sensorimotor, visual/sensory and thalamic networks (p=ranging from 0.02 to 0.04) *vs* HC; significantly higher RS FC between the IPL and the left inferior frontal gyrus *vs* HC (p=0.002) and RRMS (p=0.03).

-**RRMS patients:** lower RS FC in left cerebellar regions of the DAN (p=0.004 and 0.05) and higher RS FC between the amygdala and right middle cingulate cortex (MCC) (p=0.009) *vs* CIS.

-**SPMS patients:** significantly lower RS FC between the left cuneus and right postcentral gyrus (p=0.05) and between the right postcentral and left precentral gyrus (p=0.05) *vs* RRMS; higher RS FC between the amygdala and the right middle temporal gyrus (MTG) (p=0.04) *vs* RRMS.

-**BMS patients:** lower RS FC between the amygdala and left fusiform gyrus (p=0.02) and higher RS FC between the IPL and the left IFG of the DAN (p=0.006) *vs* RRMS.

-**PPMS patients:** significantly reduced RS FC in several parietal and cerebellar regions of the sensorimotor, visual/sensory and cerebellum networks (p=ranging from 0.001 to 0.04) *vs* HC; significantly increased RS FC between the amygdala and left putamen (p=0.03) and right MCC (p=0.007) *vs* CIS.

Effects of cognitive impairment.

-**CI MS patients**: decreased RS FC in the DMN (p=0.001) and DAN (p=0.001) *vs* CP MS. CI MS patients had also reduced RS FC of the precuneus in the DMN (p=0.05) and DAN (p=0.01), lower RS FC of the bilateral cerebellum in the DAN (p=0.04) and increased RS FC between the amygdala and right MTG (p=0.03).

-**CP MS patients**: higher RS FC between the cuneus and right MTG in the visual/sensory network *vs* HC (p=0.001) and CI patients (p=0.02).

## CONCLUSIONS

• Widespread changes of cortical/subcortical RS FC (including both dcrease and increase of RS FC) were detected in MS patients.

• Both decreased and increased RS FC abnormalities were clinically relevant, since they were correlated with patients' disability and cognitive impairment.

## REFERENCES

[1] Biswal B et al., PNAS 2010;
[2] Filippi M et al., J Neurol 2013;
[3] Sacco R et al., Mult Scler Int 2013;
[4] Tomasi D and Volkow N, Cereb Cortex 2011;
[5] Kurtzke JF, Neurology1983;
[6] Rao SM, National Multiple Sclerosis Society 1991;
[7] Amato MP et al., Mult Scler 2006;
[8] Sepulcre J et al, Mult Scler 2006.

**This work has been partially supported by a grant from Italian Ministry of Health (GR-2008-1138784/GR-2009-1529671) and Fondazione Italiana Sclerosi Multipla (FISM2012/R/8, FISM2014/R/7).**