Structural Connectivity-defined Thalamic Subregions Have Different Functional Connectivity Abnormalities in Multiple Sclerosis Patients: Implications for Clinical Correlations

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Abstract: In spite of the well-known importance of thalami in multiple sclerosis (MS), only limited data on whole and subregional thalamic functional connectivity (FC) changes are available. Using diffusion tensor imaging, we performed a structural connectivity based thalamic parcellation and investigated subregional thalamic resting-state (RS) FC alterations and their relationship with clinical/cognitive measures in MS. MRI data from a reference set of healthy controls (HC) were used to parcellate the thalami into five subregions, according to their structural connectivity. For each thalamic subregion, a seed-based RS FC analysis was performed in 187 MS patients and 94 HC. Correlations between thalamic RS FC and clinical/cognitive variables were assessed. Compared to HC, MS patients showed increased intra- and inter-thalamic RS FC for almost all thalamic subregions, and increased RS FC between all thalamic subregions and the left insula. Frontal and motor thalamic subregions also showed reduced RS FC with the caudate nucleus. For the temporal thalamic subregion, we observed reduced RS FC with the ipsilateral thalamus, anterior and middle cingulate cortex, and cerebellum. Compared to cognitively preserved, cognitively impaired MS patients had higher thalamic RS FC with several temporal areas. In MS patients, lower RS FC between thalamic subregions and the caudate and cingulate cortex correlated with worse motor performance, whereas higher RS FC with the insula correlated with better motor performance. The main thalamic subregions have different RS-FC abnormalities in MS patients. Increased thalamic RS FC with the insula may have a compensatory role, whereas

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increased RS FC with temporal areas, observed in patients with cognitive impairment may reflect maladaptive mechanisms. *Hum Brain Mapp* 38:6005–6018, 2017. © 2017 Wiley Periodicals, Inc.

Key words: thalamus; functional connectivity; parcellation; multiple sclerosis; motor performance; cognition

INTRODUCTION

A number of pathological and imaging studies have consistently demonstrated an involvement of the thalamus in patients affected by multiple sclerosis (MS) (Minagar et al., 2013). Decreased neuronal integrity, loss of neurons and macroscopic atrophy of thalamic structures have been reported from the early stages of MS, and contribute to cognitive and motor dysfunction in these patients (Alexander, et al., 1990; Harrison, et al., 2015; Minagar, et al., 2013; Wylezinska, et al., 2003).

Using functional magnetic resonance imaging (fMRI), abnormal activation and resting-state (RS) functional connectivity (FC) of the thalamus with several cortical and sub-cortical regions have also been detected. However, at present, findings on the behavior and the clinical consequences of thalamic RS-FC modifications in MS patients are still largely contradictory. Indeed, some studies showed that compared to healthy controls (HC), MS patients had increased inter- and intra-thalamic RS FC (Liu et al., 2015; Zhou et al., 2016), as well as increased RS FC between the thalami and cortical and cerebellar areas (Tona et al., 2014; Zhou et al., 2016). However, other studies described exactly the opposite, with a decreased RS FC within and between thalamic nuclei (De Giglio et al., 2016; Tona et al., 2014) and between the thalamus and cortical regions in MS patients (Liu et al., 2015; Tona et al., 2014). Concerning the clinical consequences of thalamic RS-FC abnormalities, some studies found a correlation between increased thalamo-cortical RS FC and poor cognitive performance (Tona et al., 2014), whereas others had contrary findings (De Giglio et al., 2016).

Several factors may help to explain discrepancies between studies, including differences in the clinical characteristics of patients enrolled and methods of analysis applied. Another important factor that has not been considered so far is the structural complexity of the thalamus itself and the functional subspecialization of its nuclei. Indeed, the majority of studies (De Giglio et al., 2016; Liu et al., 2015; Schoonheim et al., 2015; Tona et al., 2014; Zhou et al., 2016) have so far treated the thalamus as a single region of interest (ROI), due to problems of inferring nuclei architecture with current approaches. Anatomical connectivity studies (Behrens et al., 2003) based on the use of diffusion tensor imaging (DTI) have identified specific connections between the main thalamic nuclei and the cortex, which have a correspondence with the thalamic functional division (Johansen-Berg et al., 2005).

To test the hypothesis that the main thalamic subregions might have different RS-FC abnormalities in MS patients

in comparison to HC (thus helping to explain discrepancies in the current literature), we performed a thalamic parcellation based on thalamo-cortical structural connectivity derived from DTI, as previously described (Biseco et al., 2015), and used the thalamic subregion probability maps obtained as seed regions for the analysis of RS-FC abnormalities. The relationship between subregional thalamic RS-FC abnormalities and motor and cognitive performance in MS patients was also assessed.

METHODS

Ethics Committee Approval

Approval was received from the local ethical standards committee on human experimentation, and written informed consent was obtained from all subjects prior to enrolment.

Subjects

We screened 218 MS patients and 103 HC for inclusion in the study, taking into consideration all subjects with an MRI acquired within the last 5 years. To be included, subjects had to have undergone a complete clinical (9 MS patients excluded), neuropsychological (7 MS patients excluded) and MRI assessment. Inclusion criteria for HC were no previous history of neurological, psychiatric or cardiovascular disorders (4 HC excluded), and a normal neurological examination. Additional inclusion criteria were to: (1) be relapse- and steroid-free for at least 3 months before MRI acquisition (2 MS patients excluded); (2) have no significant medical illnesses or substance abuse that could interfere with cognitive functioning; (3) have no other major systemic, psychiatric or neurological disorders; and (4) be right-handed, according to the Edinburgh Handedness Inventory Scale (Oldfield, 1971) (5 MS patients and 4 HC excluded). These criteria lead to a selection of 195 MS patients and 95 HC. After exclusion of 8 MS and one HC due to poor image quality and/or postprocessing failure, the final cohort included 187 MS patients (136 with relapsing-remitting MS, 42 with secondary progressive MS and 9 with primary progressive MS) and 94 age- and sex-matched HC. A portion of these patients was also part of two previous investigations (Rocca et al., 2016, 2017).

Clinical and Neuropsychological Assessment

On the day of MRI scanning, all patients underwent a complete neurologic examination, with rating of the EDSS score and nine-hole peg test (9-HPT) (performed with the

right and left hand). A 9-HPT z score was calculated using normative values provided by Uitdehaag et al. (2002).

Within 48 h of the MRI scan, an expert neuropsychologist administered the Brief Repeatable Battery of Neuropsychological Tests (BRB-N) (Rao et al., 1991), which includes the Selective Reminding Test (SRT) to assess verbal memory; the 10/36 Spatial Recall Test (10/36 SRT) to assess visual memory; the Symbol Digit Modalities Test and the Paced Auditory Serial Addition Test (PASAT) 2" and 3" (Gronwall, 1977; Solari et al., 2005) to assess attention and information processing speed; and the Word List Generation test to assess verbal fluency. None of the patients had had a neuropsychological evaluation in the 6 months prior to study inclusion (thus minimizing learning effects). Patients with at least two abnormal tests, defined as a score more than 2 SD below the age- and education-corrected normative values provided by Amato et al. (2006), were considered cognitively impaired (CI).

MRI Acquisition

Using a 3.0 Tesla Philips Intera scanner (Philips Medical Systems, Eindhoven, The Netherlands), the following brain scans were acquired from all participants during a single session: (a) RS fMRI scans, using a T_2^* -weighted echoplanar imaging (EPI) sequence (TR/TE = 3,000/35 ms; flip angle = 90° , 200 sets of 30 axial slices, slice thickness = 4 mm; matrix size = 128×128 , FOV = 240×240 mm², in-plane resolution = 1.875×1.875 mm²). All subjects were instructed to keep their eyes closed, remain motionless, without thinking about anything in particular during scanning. No subject reported to have fallen asleep during scanning, according to a questionnaire delivered immediately after the MRI session; (b) pulsed-gradient SE EPI (TR/TE = 8,775/58 ms, matrix size = 112×88 , FOV = 240×231 mm², 55 contiguous, 2.3-mm thick axial slices) with SENSE (acceleration factor = 2) and diffusion gradients applied in 35 noncollinear directions. Two optimized b factors were used for acquiring diffusion weighted images ($b_1 = 0$, $b_2 = 900$ s mm⁻²); (c) dual-echo (DE) turbo SE (TR/TE = 2,599/16–80 ms, echo train length [ETL] = 6; flip angle = 90° , matrix size = 256×256 , FOV = 240×240 mm², 44 axial 3-mm-thick slices); and (d) 3D T_1 -weighted fast field echo (FFE) (TR/TE = 25/4.6 ms; flip angle = 30° ; matrix size = 256×256 ; FOV = 230×230 mm²; 220 contiguous, axial slices with voxel size = $0.89 \times 0.89 \times 0.8$ mm³).

For all scans, the slices were positioned to run parallel to a line that joined the most infero-anterior and infero-posterior margins of the corpus callosum.

MRI Analysis

T_2 hyperintense and T_1 hypointense lesion volumes (LV) were measured on DE TSE and 3D T_1 -weighted scans (after coregistration to the DE TSE sequence), respectively,

using a local thresholding segmentation technique (Jim 6, Xinapse Systems, Colchester, United Kingdom). Normalized brain (NBV), WM (WMV) and GM (GMV) volumes were measured on the 3D T_1 -weighted scans using the SIENAX software (Smith et al., 2002), after T_1 -hypointense lesion refilling (Chard et al., 2010).

Structural Segmentation of the Thalami

The thalami were segmented from the 3D T_1 -weighted images in native space using the FIRST tool from the FMRIB Software Library (Patenaude et al., 2011). This method uses shape and appearance models derived from a training set and defined in Montreal Neurological Institute (MNI) space. To bring the models into native image space, normalization is performed using a two-stage linear registration, to achieve subcortical structure alignment. To improve the segmentation, in particular for the delimitation of the boundary with the internal capsule, the script was modified to calculate both stages of the linear registration, using a single-subject fractional anisotropy (FA) map and the FA template of FSL as a reference. FA maps were derived from the tensor estimated using DW images, once corrected for movements and for distortions caused by eddy currents or induced by magnetic susceptibility variations (Bisecco et al., 2015). The results of segmentation were all visually checked. Normalized thalamic volumes were calculated from the FIRST output using the SIENAX scaling factor (Smith et al., 2002).

Segmentation of thalamic subregions was obtained by applying a previously derived MNI space atlas (Bisecco et al., 2015) that includes probability maps of thalamic parcellation according to the bilateral structural connectivity of each thalamic voxel in frontal, motor, postcentral, temporal and occipital cortical regions. For all subjects studied, the thalamic subregion probability maps were transformed to the RS fMRI images, concatenating the nonlinear deformation between the MNI space and the 3D T_1 -weighted image (FNIRT tool), the linear transformation to the T_2 -weighted image (FLIRT tool) and the nonlinear transformation between the scalped T_2 -weighted image and the mean RS fMRI map.

Finally, the thalamic subregion of interest, as well as the whole-thalamus ROI, were used as a mask and applied to RS fMRI scans of each subject to perform a seed-based analysis of RS FC.

FMRI Analysis

FMRI data were analyzed using Statistical Parametric Mapping (SPM) 12 (<http://www.fil.ion.ucl.ac.uk/spm/>) and REST software (<http://resting-fmri.sourceforge.net>). First, each subject's images were realigned to the mean of all that subject's images to correct for motion (7 MS patients excluded for excessive motion [i.e., in case of a maximum cumulative translation/rotation > 4 mm/5 degrees]; mean framewise displacement for the remaining

TABLE I. Main demographic, clinical, and conventional MRI characteristics of the healthy controls (HC) and multiple sclerosis (MS) patients

	HC (<i>n</i> = 94)	MS patients (<i>n</i> = 187)	<i>P</i>
Mean age, years (SD)	41.5 (14.6)	40.8 (12)	0.76 ^a
Male/Female	46/48	80/107	0.76 ^b
Mean disease duration, years (SD)	–	13.3 (8)	–
Median EDSS (range)	–	2.0 (0.0–8.0)	–
Mean education, years (SD)	13.5 (3)	12.8 (3)	0.89
CP/CI	–	122/65	–
Mean 9-HPT <i>z</i> -score (SD)	–0.1 (1)	–2.42 (1)	<0.001 ^a
Mean T ₂ LV (SD) (mL)	–	9.9 (10)	–
Mean T ₁ LV (SD) (mL)	–	7.1 (8)	–
Mean NBV (SD) (mL)	1577 (84)	1498 (104)	<0.0001 ^a
Mean GMV (SD) (mL)	736 (57)	684 (74)	<0.0001 ^a
Mean WMV (SD) (mL)	841 (43)	814 (48)	<0.0001 ^a
Mean nRThalV (SD) (mL)	10.3 (0.7)	9.3 (1.1)	<0.0001 ^a
Mean nLThalV (SD) (mL)	10.6 (0.8)	9.6 (1.1)	<0.0001 ^a

^aMann-Whitney test.^bPearson chi-square test.

List of abbreviations: SD = standard deviation; EDSS = expanded disability status scale; CP = cognitively preserved; CI = cognitively impaired; LV = lesion volume; NBV = normalized brain volume; GMV = gray matter volume; WMV = white matter volume; nRThalV = normalized right thalamic volume; nLThalV = normalized left thalamic volume.

subjects: 0.14 mm, SD = 0.08, and 0.17 mm, SD = 0.11 for controls and MS patients, $P = 0.06$). Then, linear trends were removed and temporal filtering (0.01 Hz < f < 0.08 Hz) was applied. Masks for CSF and white matter (WM) were created using SPM12 and FIRST segmentations. In particular, SPM12 was used to obtain CSF and WM probability maps, and FIRST segmentations were used to remove from WM maps any residual deep GM structures. These masks were used to extract mean WM and CSF signal time courses. Non-neuronal sources of synchrony between RS fMRI time series and motion-related artifacts were minimized by regressing out the six motion parameters estimated by SPM12, as well as the mean WM and CSF signals. After Gaussian smoothing (6 mm³ full-width at half maximum), maps of RS FC were obtained for each subject using a seed-region correlation approach (Biswal et al., 1995). Seeds were selected from the whole thalamus and from the thalamic subregion masks previously registered. RS FC calculation was performed in the native subject space to increase the overlay between seeds and RS fMRI images. Correlation coefficients were converted to *z* values using Fisher's *r*-to-*z* transform to improve normality (Song et al., 2011). Finally, before statistical analysis, thalamic RS FC *Z* maps were (1) coregistered to the 3D T₁-weighted scan using FSL boundary based registration; and (2) normalized to the MNI space using FSL FLIRT and FNIRT registrations.

Statistical Analysis

Between-group comparisons of demographic, clinical and conventional MRI variables were performed using Pearson Chi-square test and the Mann-Whitney test as

appropriate (SPSS software, version 23.0). Skewed distributed variables were log-transformed before analyses.

Using SPM12, three separate second level analyses were carried out on thalamic RS FC maps: (1) average within-group RS FC in HC and MS patients, separately (one sample *t* tests); (2) between-group comparison of RS FC between HC, cognitively preserved (CP) and CI MS patients (ANOVA models, with age and disease duration included as nuisance variables); and (3) correlation of thalamic RS FC with clinical, neuropsychological and 9-HPT scores (multiple regression models, age and disease duration adjusted, masked for the difference between MS patients and HC). All second-level analyses were tested at $P < 0.05$, family-wise error (FWE) corrected for multiple comparisons. Given the exploratory nature of this study, due to the application of a relatively new approach to perform thalamic FC at subregional level, clusters surviving at $P < 0.001$ (uncorrected, cluster extent threshold $k = 10$) were also identified.

RESULTS

The main demographic, clinical, and MRI characteristics of all study participants are summarized in Table I. Compared to HC, MS patients had lower NBV, GMV, WMV, and thalamic volumes ($P < 0.0001$).

Sixty-five (34.8%) MS patients were classified as CI. The most frequently involved cognitive domains were: attention and information processing speed (19.3% of the patients), executive functions (15% of the patients), verbal memory (21.9% of the patients), spatial memory (5.3% of the patients) and verbal fluency (15% of the patients).

Figure 1.

Thalamic subregions individually used as seed regions for the resting state (RS) functional-connectivity (FC) analysis (first column) and the corresponding seed-based RS FC maps in healthy controls (HC) (second column; $P < 0.001$) and patients with multiple sclerosis (MS) (third column; $P < 0.001$). Images are

presented in the neurological convention. All RS FC maps are from left sided seed regions. See text for further details. List of abbreviations: SR = subregion; L = left; R = right. [Color figure can be viewed at wileyonlinelibrary.com]

Thalamic RS FC

Both in MS patients and HC, each thalamic subregion RS-FC map partially resembled structural connections with cortical areas previously described (Bisecco et al., 2015) (Fig. 1).

The results of the comparisons between MS patients and HC are summarized in Table II. Using the whole thalamus as a seed region for the analysis, compared to HC, MS

patients had reduced FC between the thalamus and the bilateral caudate nucleus as well as increased intra-thalamic RS FC and increased RS FC with the left insular cortex.

The analysis of thalamic subregion RS FC showed that, in comparison to HC, in MS patients all thalamic subregions had an increased RS FC with the left insular cortex (Table II; Fig. 2). Apart the temporal thalamic subregion, all remaining subregions also showed increased intra- and

TABLE II. Brain regions with thalamic resting state (RS) functional-connectivity differences between multiple sclerosis (MS) patients and healthy controls (HC) ($P < 0.001$; cluster extent $k = 10$ voxels)

Thalamic seed region	Connected region	Side	MNI coordinates (<i>x, y, z</i>)			<i>k</i> extent	<i>t</i> value
Reduced thalamic RS FC in MS patients compared to HC							
Whole left thalamus	Caudate nucleus	R	10	10	12	142	4.78
	Caudate nucleus	L	−10	22	2	95	4.34
Whole right thalamus	Caudate nucleus	R	8	12	12	48	4.51
	Caudate nucleus	L	−10	10	16	28	3.89
Left frontal SR	Caudate nucleus	L	−8	20	2	43	3.94
Left motor SR	Caudate nucleus	L	−10	22	2	17	3.59
Left temporal SR	Thalamus	L	−8	−10	4	93	4.72
	Thalamus	R	2	−18	0	93	4.75
Right temporal SR	Caudate nucleus	R	10	10	12	31	4.09
	Anterior cingulum	R	4	42	4	27	3.49
	Cerebellum	R	46	−70	−42	213	4.31 ^a
	Thalamus	R	8	−10	2	10	4.94
	Middle cingulum	R	4	34	38	73	4.49
	Cerebellum	L	−48	−72	−38	786	5.16 ^a
Increased thalamic RS FC in MS patients compared to HC							
Whole left thalamus	Thalamus	L	−8	−24	−2	23	4.37
	Insula	L	−39	7	−4	19	3.85
Whole right thalamus	Thalamus	R	14	−24	12	106	5.52
	Insula	L	−32	14	5	25	4.05
Left frontal SR	Thalamus	L	−14	−24	14	45	4.84
	Insula	L	−32	−22	20	29	3.91
Right frontal SR	Thalamus	R	14	−22	12	151	6.21
	Thalamus	L	−2	−16	10	151	3.89
Left motor SR	Thalamus	L	−14	−22	12	178	8.61 ^a
	Thalamus	R	14	−22	12	71	4.88
Right motor SR	Insula	L	−32	−22	20	156	5.58
	Thalamus	R	14	−22	10	336	7.18 ^a
	Thalamus	L	−14	−24	14	336	5.35 ^a
Left occipital SR	Insula	L	−32	−22	20	80	5.09
	Thalamus	L	−16	−30	8	241	8.30 ^a
	Thalamus	R	14	−24	12	187	5.93 ^a
Right occipital SR	Insula	L	−32	−22	20	37	4.62
	Thalamus	R	14	−28	8	432	10.05 ^a
	Thalamus	L	−6	−26	8	432	5.68 ^a
Left postcentral SR	Supramarginal gyrus	R	52	−44	32	284	3.84
	Thalamus	L	−16	−26	10	224	9.25 ^a
	Thalamus	R	14	−24	10	154	5.65
Right postcentral SR	Insula	L	−32	−22	20	93	5.48
	Thalamus	R	16	−26	8	381	8.16 ^a
	Thalamus	L	−16	−24	12	381	5.47 ^a
Left temporal SR	Insula	L	−32	−22	20	111	5.05
	Precuneus	R	14	−60	62	16	3.47
	Right temporal SR	Insula	L	−34	−16	14	170
Precuneus		L	−6	−40	70	24	3.50

^aClusters significant after family-wise error correction ($P < 0.05$).

List of abbreviations: SR = subregion; L = left; R = right; MNI = Montreal Neurological Institute.

inter-thalamic RS FC. Compared to HC, in MS patients reduced RS FC was found between the left frontal and left motor thalamic subregion and left caudate nucleus as well as between the temporal thalamic subregion and the ipsilateral thalamus, cingulate cortex, and contralateral cerebellum (Table II; Fig. 2).

The results of the comparisons between CP and CI MS patients and HC, separately, are summarized in Table III and shown in Figure 3. Compared to CP, CI MS patients showed increased RS FC between several thalamic subregions (frontal, motor, occipital, and temporal subregion) and temporal areas, such as the hippocampus,

Figure 2.

Results of the group comparison of thalamic resting-state (RS) functional connectivity (FC) between patients with multiple sclerosis (MS) and healthy controls (HC) ($P < 0.001$ uncorrected; cluster extension threshold = 10 voxels). Images are presented in the neurological convention. All results showed are from left sided seed regions of whole thalamus and thalamic subregions. See text and Table II for further details. List of abbreviations: SR = subregion; L = left; R = right. [Color figure can be viewed at wileyonlinelibrary.com]

parahippocampal gyrus, and superior temporal cortex (Table III; Fig. 3).

Analysis of Correlations

The results of the analysis of correlation between thalamic RS FC abnormalities and clinical variables in MS patients are summarized in Table IV. Significant correlations ($P < 0.001$) were found between:

- worse 9-HPT performance and lower RS FC between thalamic subregion and caudate nuclei, cingulate cortex and cerebellum (Table IV; Fig. 4);
- better 9-HPT performance and higher RS FC of thalamic subregions with the insula (Table IV; Fig. 4);
- higher EDSS score and lower RS FC of thalamic subregion with caudate nuclei, supramarginal gyrus and cerebellum (Table IV).

No correlation was found between thalamic RS-FC abnormalities and T2 LV.

DISCUSSION

Using a seed-based approach to evaluate thalamic RS FC in a relatively large cohort of MS patients, we found that thalamic subregions, identified according to their structural connectivity with the cerebral cortex, had different RS-FC abnormalities. In particular, in MS patients compared to HC, all thalamic subregions, except the temporal region, showed increased intra- and inter-thalamic RS FC. The left frontal and motor thalamic subregions of MS patients showed reduced RS FC with the ipsilateral caudate nucleus. The temporal thalamic subregion behaved differently, showing reduced intra- and inter-thalamic RS FC as well as reduced RS FC with the cingulate cortex and contralateral cerebellum.

Such results are in agreement with those from previous studies that showed an increased intra-thalamic RS FC in MS patients compared to HC (Liu et al., 2015; Zhou et al., 2016). However, other studies (De Giglio et al., 2016; Tona et al., 2014) reported a decreased intra- and inter-thalamic RS FC, a finding observed in our MS cohort when the temporal thalamic subregion was used as the seed region for the fMRI analysis. Considering thalamic RS FC with cortical areas in MS patients, there is a lot of heterogeneity in previous literature. Tona et al. (2014) reported a higher RS FC between the thalami and cerebellum, basal ganglia, hippocampus, cingulum, temporo-occipital, insular, frontal, and parietal cortices in MS patients. In the same study, they also reported a decreased RS FC in MS patients between the thalami and other regions in the cerebellum, cingulum, insular, prefrontal and parieto-occipital cortices (Tona et al., 2014). In 35 MS patients, Liu et al. (2015) described a decreased RS FC between the thalami and several brain regions, including the right middle frontal and parahippocampal gyri, and the left inferior parietal lobule.

Figure 3.

Results of the comparison of thalamic resting-state (RS) functional connectivity (FC) between cognitively impaired (CI) and cognitively preserved (CP) MS patients (first column), between CI MS patients and healthy controls (HC) (second column) and between CP MS patients and HC (third column) ($P < 0.001$ uncorrected; cluster extension threshold = 10 voxels). Images

are presented in the neurological convention. All results showed are from left sided seed regions of whole thalamus and thalamic subregions. See text and Table III for further details. List of abbreviations: SR = subregion; L = left; R = right. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE III. Brain regions with thalamic resting state (RS) functional-connectivity differences between multiple sclerosis (MS) patients with and without cognitive impairment and healthy controls (HC) ($P < 0.001$; cluster extent $k = 10$ voxels)

Thalamic seed-region	Connected region	Side	MNI coordinates (x, y, z)			k extent	t value
Increased thalamic RS FC in CI compared to CP MS patients							
Whole left thalamus	Hippocampus	L	-18	-6	-20	143	4.27
	Parahippocampal Gyrus	L	-16	2	22	143	3.69
Whole right thalamus	Parahippocampal Gyrus	L	-12	4	-20	247	4.57
	Parahippocampal Gyrus	R	18	2	-26	139	4.06
	Hippocampus	L	-18	-34	4	107	4.31
Left frontal SR	Hippocampus	L	-18	-6	-20	83	3.93
Right frontal SR	Hippocampus	L	-18	-34	4	49	3.87
	Hippocampus	R	18	-34	2	86	3.81
Left motor SR	Parahippocampal Gyrus	R	18	6	-24	16	3.65
Right motor SR	Parahippocampal Gyrus	R	16	6	-20	174	3.94
	Hippocampus	L	-18	-36	6	12	3.48
Left occipital SR	Hippocampus	L	-18	-6	-14	30	3.70
Right occipital SR	Parahippocampal Gyrus	R	18	8	-18	50	4.05
Left temporal SR	Hippocampus	L	-18	-4	-20	327	4.95 ^a
	Parahippocampal Gyrus	L	-12	4	-20	327	3.76 ^a
	Superior Temporal Gyrus	R	64	-12	0	68	4.51
Right temporal SR	Superior Temporal Gyrus	R	50	6	-10	1314	5.18 ^a
Increased thalamic RS FC in CI MS patients compared to HC							
Whole left thalamus	Thalamus	L	-8	-30	8	37	3.75
Whole right thalamus	Thalamus	R	14	-24	12	184	5.61 ^a
Left frontal SR	Thalamus	L	-14	-24	14	15	4.41
Right frontal SR	Thalamus	R	14	-24	12	138	5.69 ^a
	Hippocampus	L	-34	6	27	25	4.22
Left motor SR	Thalamus	L	-14	-22	12	181	7.77 ^a
	Thalamus	R	14	-22	12	55	4.63
	Insula	L	-32	-22	20	78	4.55
Right motor SR	Thalamus	R	14	-22	10	135	6.43 ^a
	Thalamus	L	-14	-24	14	14	4.48
	Hippocampus	L	-14	-34	6	28	4.01
Left occipital SR	Thalamus	L	-16	-30	8	259	8.24 ^a
	Thalamus	R	14	-24	12	251	5.74 ^a
	Insula	L	-32	-22	20	75	4.36
Right occipital SR	Thalamus	R	14	-28	8	458	9.09 ^a
	Thalamus	L	-16	-32	6	59	4.95
	Insula	R	42	2	-12	18	3.98
Left temporal SR	Hippocampus	L	-16	-34	8	92	4.48
	Insula	R	42	4	-12	29	4.31
	Precuneus	R	10	-58	64	33	3.45
Right temporal SR	Thalamus	R	12	-30	10	159	5.50 ^a
	Hippocampus	R	20	-32	10	56	4.75
	Insula	L	-38	-2	-12	593	5.02 ^a
	Insula	R	42	2	-12	183	4.95 ^a
	Hippocampus	L	-18	-34	6	92	4.63
Increased thalamic RS FC in CP MS patients compared to HC							
Whole left thalamus	Insula	L	-32	-20	20	104	3.85
Whole right thalamus	Thalamus	R	14	-24	10	20	3.85
Left frontal SR	Thalamus	L	-12	-22	14	37	4.01
	Insula	L	-32	4	14	10	3.78
	Insula	R	34	8	14	30	4.09
Right frontal SR	Thalamus	R	10	-20	12	116	5.46 ^a
	Thalamus	L	-4	-18	10	55	3.91
	Insula	L	-32	-10	16	51	3.63

TABLE III. (continued).

Thalamic seed-region	Connected region	Side	MNI coordinates (x, y, z)			k extent	t value
Left motor SR	Thalamus	L	-14	-22	12	126	7.11 ^a
	Thalamus	R	8	-22	8	40	3.90
	Insula	L	-32	-22	20	153	5.18 ^a
	Insula	R	34	10	14	25	3.45
Left occipital SR	Thalamus	L	-18	-28	10	98	6.45 ^a
	Thalamus	R	14	-22	12	61	4.55
Right temporal SR	Thalamus	R	10	-28	12	111	4.64
	Hippocampus	R	20	-32	10	25	3.94

^aClusters significant after family-wise error correction ($P < 0.05$).

SR = subregion; L = left; R = right; MNI = Montreal Neurological Institute.

A mixed pattern of increased and decreased thalamic RS FC was also found by Zhou et al. (2016), who showed significantly increased RS FC between the thalamus and the primary motor cortex, occipital, prefrontal and temporal cortex in RRMS patients, as well as a decreased RS FC between the thalamus and other regions of the pre-frontal cortex. In a prospective study of 24 RRMS patients, De Giglio et al. (2016) showed lower RS FC in the thalamic RS network of the patients who, compared to HC, exhibited

significantly lower RS FC of clusters located bilaterally in the cerebellum, caudate nuclei, frontal and occipital cortices.

Such a heterogeneity of results can be partially explained by our findings, showing variable RS FC patterns among the main thalamic subregions. In our study, indeed, the RS FC analysis performed using the whole thalamus as a seed region, was not able to detect the different patterns of subregional thalamic RS FC that were hidden by the whole thalamus effect. Specifically, the temporal thalamic subregion had a pattern of RS FC abnormalities that diverged from that of the whole thalamus and the other thalamic subregions, with a reduced inter- and intra-thalamic RS FC and an increased RS FC with posterior areas such as precuneus. Although not significantly correlated with motor or cognitive performance, these alterations reflect different patterns of RS FC of thalamic subregions. The different behavior of thalamic subregions can be explained by the complex organization of this structure, composed of GM nuclear groups interconnected by WM fascicles (Hannoun et al., 2012), and the uneven distribution of MS-related damage in these two compartments. In line with this, a previous thalamic parcellation study (Bisecco et al., 2015), performed on a different group of subjects, found that, except for the temporal subregion, all remaining thalamic subregions had increased fractional anisotropy (FA) in MS patients, which was interpreted as the possible expression of more prominent GM damage. In contrast to the other thalamic subregions, the temporal region showed a tendency towards a reduction of FA (Bisecco et al., 2015), which may reflect the presence of intra-thalamic WM damage in thalamic subregions with a higher proportion of WM (Hannoun et al., 2012).

In our study, the only finding shared by all thalamic subregions in MS patients was the increased RS FC with the left insular cortex, which was related to better motor performance. On the other hand, the reduced RS FC between some thalamic subregions (left frontal, left motor, temporal) with caudate, cingulate cortex, supramarginal gyrus and cerebellum was related to worse motor performance. These results highlight the role of the left insular cortex as a region where adaptive mechanisms are involved in the preservation of motor performance. In healthy controls, the insular cortex has been involved in perceived ownership of a body part (Tsakiris et al., 2007)

TABLE IV. Correlations between thalamic resting state (RS) functional-connectivity (FC) abnormalities and clinical variables (9-HPT and EDSS) in MS patients ($P < 0.001$)

Thalamic seed region	Connected region	9-HPT z-score	EDSS
Whole left thalamus	L Caudate nucleus	0.34 ^a	-0.45 ^a
	R Caudate nucleus	0.32 ^a	-0.42 ^a
	L Insula	0.29 ^a	-
	R Insula	0.28 ^a	-
Whole right thalamus	L Caudate nucleus	0.38 ^a	-0.44 ^a
	R Caudate nucleus	0.34 ^a	-0.33 ^a
	L Insula	0.35 ^a	-
Left frontal SR	L Caudate nucleus	0.34 ^a	-0.46 ^a
	R Caudate nucleus	0.33 ^a	-0.45 ^a
	L Insula	0.30 ^a	-
	R Insula	0.33 ^a	-
Left motor SR	L Insula	0.29	-
Right motor SR	L Insula	0.34 ^a	-
	R Insula	0.31 ^a	-
Right postcentral SR	L Insula	0.29	-
Right occipital SR	R Supramarginal	-	-0.36 ^a
Left temporal SR	L Caudate nucleus	0.37 ^a	-0.40 ^a
	R Caudate nucleus	0.34	-
	R Anterior cingulum	0.30	-
	L Cerebellum	0.30	-0.41 ^a
Right temporal SR	R Cerebellum	0.30	-0.39 ^a
	R Middle cingulum	0.32	-
	R Cerebellum	-	-0.34

^aCluster significant after family-wise error correction ($P < 0.05$).

List of abbreviations: SR = subregion; 9-HPT = nine-hole peg test; EDSS = expanded disability status scale; L = left; R = right.

Figure 4.

Regions of significant correlations between motor performance (measured by 9-HPT z scores) and thalamic resting-state (RS) functional connectivity (FC) in MS patients showed in coronal plane (first column) and axial plane (second column) ($P < 0.001$ uncorrected; cluster extension threshold = 10 voxels). The third

column shows scatterplots of the strongest correlations for each thalamic subregion. Images are presented in the neurological convention. See text and Table IV for further details. List of abbreviations: SR = subregion; L = left; R = right. [Color figure can be viewed at wileyonlinelibrary.com]

and self-recognition (Devue et al., 2007), functions that are implicated in motor planning and execution. In diseased patients, the insular cortex has been implicated in motor improvement after a motor stroke (Weiller et al., 1993) and MS relapse (Pantano et al., 2002). The reduction of RS FC between the thalami and caudate nuclei, cingulate cortex and cerebellum seems to have an important role in determining motor impairment in MS, in line with the results of a recent study (Rocca et al., 2017).

Exploring the relationship between cognitive performance in MS patients and thalamic RS FC, Tona et al. (2014) showed an association between decreased cognitive performance and increased thalamic RS FC with the thalamus, cerebellum, hippocampi and some cortical areas in the frontal, temporal, parietal, and occipital lobes, bilaterally. Analogously, in patients with severe cognitive impairment, Schoonheim et al. (2015) found an increased thalamic RS FC with sensorimotor, frontal and occipital brain regions. Moreover, previous

studies that explored large-scale brain networks by means of either fMRI or magnetoencephalography, reported an association between increased RS FC and cognitive decline in MS (Hardmeier et al., 2012; Hawellek et al., 2011).

Partially in agreement with these previous findings, in our study the analysis of abnormalities of thalamic RS FC according to the presence/absence of cognitive impairment demonstrated that compared to CP, CI MS patients had increased RS FC between almost all thalamic subregions (frontal, motor, occipital and, most prominently, temporal) and several temporal areas, such as the hippocampus, parahippocampal gyrus, and superior temporal cortex, possibly reflecting a maladaptive mechanism leading to a worse cognitive performance. Such thalamic RS-FC abnormalities, indeed, may be related to functional abnormalities between cortical and thalamic structures, as a compensatory mechanism due to brain plasticity and functional remapping (Grady et al., 2001; Liu et al., 2015), which, in some cases, can result in maladaptive mechanisms (Gardini et al., 2015). On the basis of this knowledge, our results suggest that there are different patterns of thalamic RS FC in MS patients partially driven by different thalamic subregions: some alterations, typified by increased thalamic FC with the left insular cortex, may have a compensatory role, while others, typified by increased thalamic FC with temporal regions, may be maladaptive, leading to a worsening of cognitive performance.

Our study has some limitations. First, it is cross-sectional. Longitudinal studies are required to clarify the dynamic relationship between thalamic RS FC abnormality accrual and clinical manifestations in MS. Second, we limited our analysis to RS fMRI, T₂ brain lesions and atrophy. As a consequence, we cannot exclude the possibility that the inclusion of other MRI techniques, such as imaging of GM lesions, would have allowed the detection of other processes contributing to clinical dysfunction in these patients. Thirdly, thalamic parcellation was performed transforming to native space the probability maps derived from a group of HC in the MNI space. This assumes that different degrees of atrophy of each thalamic subregion could be correctly accounted for by the nonlinear registration method, modelling atrophy inside the thalamus from atrophy detected at the borders. This assumption might not always be correct and some degree of non-uniform atrophy could remain not compensated. Also, the resolution of RS-fMRI maps is lower than that of 3D T₁-weighted images, used for thalamic parcellation, and DTI maps, used to produce connectivity-based parcellation. However, considering the regularity of thalamic shape and the low degree of atrophy in our MS group, we can assume that the further down-sampling needed to match RS-fMRI resolution, does not compromise the detection of fMRI signal from different thalamic subregions. Fourth, motor and cognitive performance were quantified by selecting a subset of the available clinical scales (9-HPT, EDSS, BRB-N) that have a proven role in measuring motor (Kragt et al., 2006;

Kurtzke, 1983) and cognitive (Portaccio et al., 2009) deficits in these patients. As a consequence, our results need to be replicated with the inclusion of a more comprehensive assessment of clinical dysfunction. Fifth, although we employed state-of-the art methods for RS fMRI-data post-processing and removal of motion-related and physiological-related artefacts, it is likely that the use of more sophisticated techniques (such as retrospective correction of images using physiological recordings, or denoising based on independent component analysis) might have altered the statistical significance of our results, as shown by recent studies of the default mode network (Griffanti et al., 2015) (hypothesizing that a similar effect would occur with thalamic seeds).

In conclusion, our study identified in MS patients different patterns of RS FC among thalamic subregions, confirming our working hypothesis. The divergent behavior of the temporal-thalamic RS network, compared to other thalamic RS networks, may help to explain the variability of thalamic RS FC findings in previous studies.

CONFLICTS OF INTEREST

A d'Ambrosio, M Hidalgo de la Cruz, P Valsasina, E Pagani, B Colombo, M Rodegher have nothing to disclose.

G Comi has received consulting fees for participating on advisory boards from Novartis, Teva Pharmaceutical Ind. Ltd, Sanofi, Genzyme, Merck Serono, Bayer, Actelion and honoraria for speaking activities for Novartis, Teva Pharmaceutical Ind. Ltd, Sanofi, Genzyme, Merck Serono, Bayer, Biogen, Excemed.

M Filippi is Editor-in-Chief of the Journal of Neurology; serves on a scientific advisory board for Teva Pharmaceutical Industries; has received compensation for consulting services and/or speaking activities from Biogen Idec, ExceMED, Novartis, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Teva Pharmaceutical Industries, Novartis, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, Cure PSP, Alzheimer's Drug Discovery Foundation (ADDF), the Jacques and Gloria Gossweiler Foundation (Switzerland), and ARiSLA (Fondazione Italiana di Ricerca per la SLA).

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