

Hippocampal-DMN Disconnectivity in MS is Related to WM Lesions and Depression

Maria A. Rocca,^{1,2} Emanuele Pravata,^{1,3} Paola Valsasina,¹ Marta Radaelli,² Bruno Colombo,² Laura Vacchi,¹ Claudio Gobbi,⁴ Giancarlo Comi,² Andrea Falini,⁵ and Massimo Filippi^{1,2*}

¹*Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy*

²*Department of Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy*

³*Department of Neuroradiology, Neurocenter of Southern Switzerland, Civic Hospital, Lugano, Switzerland*

⁴*Department of Neurology, Neurocenter of Southern Switzerland, Civic Hospital, Lugano, Switzerland*

⁵*Department of Neuroradiology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milan, Italy*

Abstract: The hippocampus is part of the default-mode network (DMN) and is functionally hit early in multiple sclerosis (MS). Hippocampal and DMN dysfunctions have been associated with depression, both in patients with MS and in major depressive disorders. We hypothesized that white matter lesions may contribute, through a disconnection mechanism, to hippocampal dysfunction. To test this, we assessed the relationship between hippocampal resting-state (RS) functional connectivity (FC) abnormalities with brain T2 lesion volumes and the presence and severity of depression. Structural and RS fMRI images were acquired from 69 patients with cognitively intact MS and 42 matched healthy controls (HC). Depression was quantified using the Montgomery–Asberg Depression Rating Scale. Seed-voxel hippocampal RS FC was assessed. SPM8 was used for between-group comparisons and correlation analysis between RS FC abnormalities with clinical and structural MRI variables.

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*Correspondence to: Prof. Massimo Filippi, Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Via Olgettina, 60, 20132 Milan, Italy. E-mail: filippi.massimo@hsr.it

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Compared to HC, patients with MS showed a significant atrophy of the whole brain and left hippocampus ($P < 0.001$), and a distributed pattern of decreased RS FC between the hippocampi and several cortical–subcortical regions, which were mostly located within the DMN. Reduced hippocampal RS FC with regions of the DMN was strongly correlated with higher T2 lesion volume, longer disease duration, and the severity of depression and disability. In patients with cognitively preserved MS, brain focal WM lesions are related to the functional integration of the hippocampus to other brain regions of the DMN, suggesting a disconnection syndrome. Such a disruption of hippocampal RS FC is likely to contribute to the occurrence of depression and to clinical disability. *Hum Brain Mapp* 36:5051–5063, 2015. © 2015 Wiley Periodicals, Inc.

Key words: hippocampus; default-mode network; resting-state fMRI; multiple sclerosis; WM lesions; depression

INTRODUCTION

Beyond focally demyelinated lesions in the white matter (WM), damage to the gray matter (GM) is known to occur from the earliest clinical stages of multiple sclerosis (MS) [Geurts and Barkhof, 2008; Kutzelnigg et al., 2005]. Such a damage contributes to some of the clinical manifestations of the disease, including cognitive deficits [Rovaris et al., 2000]. As part of the archeo-cortex, the hippocampus is another target of demyelination and neuronal loss, which typically take place in MS [Geurts et al., 2007; Papadopoulos et al., 2009]. Hippocampal involvement in MS is clinically relevant, since it has been associated to deficits of verbal and visuospatial memory [Longoni et al., 2015; Roosendaal et al., 2008; Sicotte et al., 2008]. Studies in patients with major depressive disorders have also suggested that the hippocampus is likely involved in the pathogenesis of depression, as shown by a hyperactivity of the hypothalamic–pituitary–adrenal (HPA) axis [Pariante and Lightman 2008], impaired neurogenesis [Miller and Hen, 2015], and reduced hippocampal volumes [Koolschijn et al., 2009]. Depression also affects more than 50% of patients with MS and has been associated with structural hippocampal abnormalities [Gold et al., 2010, 2014; Kiy et al., 2011].

Resting-state (RS) functional MRI (fMRI) is a task-free technique which provides an unbiased assessment of the architecture and integrity of brain functional networks by evaluating spontaneous fluctuations of the blood-oxygen-level-dependent (BOLD) signal occurring at rest [Biswal et al., 1995; Cordes et al., 2000]. The application of RS fMRI methods is contributing to provide valuable insights into the pathophysiological mechanisms of several neurological and psychiatric conditions [Greicius, 2008; Greicius et al., 2007; Rocca et al., 2010, 2014]. In this context, the intrinsic activity of the default-mode network (DMN), a medial cortical network involving several fronto-parietal brain regions and the hippocampus, which is active at rest, and deactivated during performance of goal-oriented tasks, is of particular interest [Raichle et al., 2001]. In patients with major depressive disorders, DMN abnormalities have been shown [Bluhm et al., 2009; Chen et al., 2015; Zeng et al., 2012; Zhu et al., 2012] and have been

linked to aberrant psychological processes occurring in this condition, such as alterations of self-referential schemes, cognitive biases, ruminations, and processing mode (over-general vs. concrete) [Belzung et al., 2015]. An abnormal RS functional connectivity (FC) of the hippocampus with frontal, temporal, and parietal regions has also been demonstrated in these patients [Cao et al., 2012; Shu et al., 2014; Zeng et al., 2012]. Interestingly, abnormalities of the DMN and hippocampal/parahippocampal regions had the highest discriminative power in classifying major depressive individuals vs. healthy controls [Zeng et al., 2012]. At present, only two studies assessed hippocampal RS FC in MS [Hulst et al., in press; Roosendaal et al., 2010] and showed abnormal hippocampal connectivity not only in patients with memory deficits [Hulst et al., in press], but also in those with minimal hippocampal structural damage and intact spatial memory functions [Roosendaal et al., 2010]. To date, there are no studies relating modifications of hippocampal connectivity with depressive symptoms in patients with MS.

In this study, we wished to gain some additional insights into the mechanisms leading to hippocampal dysfunction in MS and its relevance for depression. Our working hypothesis was that WM focal lesions may contribute, through a disconnection mechanism, to hippocampal dysfunction and depression. To test this, we assessed the relationship between abnormalities of hippocampal RS FC with brain T2 lesion volumes (LV) and depression in a relatively large cohort of patients with MS. Since disability has been associated with more severe depression [Chwastiak et al., 2002], we also investigated the correlation between hippocampal disconnectivity and the Expanded Disability Status Scale (EDSS) score.

MATERIALS AND 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 study enrolment.

Subjects

Sixty-nine consecutive relapse-onset patients with MS and 42 gender- and age-matched healthy controls (HC) were enrolled. All HC had to have no previous history of neurological, psychiatric, or cardiovascular disorders, and a normal neurological examination. To be included, patients had to be relapse- and steroid-free in the previous three months; no significant medical illnesses or substance abuse that could interfere with cognitive functioning; no other major systemic, psychiatric, or neurological diseases. To avoid possible confounding effects on the interpretation of our analysis of correlation with depression, patients with fatigue (defined as a score ≥ 4 at the Fatigue Severity Scale) [Krupp et al., 1989] and cognitive impairment were excluded from the study. Cognitive impairment was evaluated based on the performance at the Brief Repeatable Battery of Neuropsychological Tests [Rao, 1990]. Performance at each test of the battery was considered pathological if patients' score was < 2 SD compared to the Italian normative data [Amato et al., 2006]. To be included, patients had to have abnormalities at less than 2 tests of the battery.

Clinical Assessment

On the same day of MRI acquisition, neurological disability was rated by an experienced observer, blinded to MRI data, using the EDSS. Depressive symptoms were assessed in all patients by administering the Montgomery–Asberg Depression Rating Scale (MADRS) [Montgomery and Asberg, 1979], a standardized measure of mood disorders, which has been previously used in MS [Amato et al., 2006; Gobbi et al., 2013]. Cumulative scores range from 0 (no depression) to 60 (severe depression).

MRI Acquisition

Using a 3.0 Tesla Philips Intera scanner, the following brain sequences were acquired from all study subjects: (a) dual-echo (DE) turbo spin echo (TSE) (repetition time (TR)/echo time (TE) = 2,599/16–80 ms; echo train length = 6; flip angle = 90° , 44 contiguous, 3-mm-thick, axial slices, matrix size = 256×256 , field of view [FOV] = 240×240 mm²); (b) 3D T1-weighted fast field echo (FFE) (TR/TE = 25/4.6 ms, flip angle = 30° , 220 contiguous, axial slices, voxel size = $0.89 \times 0.89 \times 0.8$ mm³, matrix size = 256×256 , FOV = 230×230 mm²); and (c) T2*-weighted single-shot echo planar imaging (EPI) sequence for RS fMRI (TR = 3,000 ms, TE = 35 ms, flip angle = 90° , FOV = 240 mm², matrix = 128×128 , slice thickness = 4 mm, 200 sets of 30 contiguous axial slices after automatic discard of the first two images, parallel to the AC–PC plane, with a total acquisition duration of about 10 min). All subjects were instructed to remain motionless, without thinking anything in particular during scanning. No subject reported to be fallen asleep during

scanning, according to a questionnaire delivered immediately after the MRI session.

Structural MRI Analysis

T2 hyperintense and T1 hypointense LV were quantified using a local thresholding segmentation technique (Jim 5, Xinapse Systems Ltd., Northants, UK).

SIENAX and FIRST (version 2.6, both part of FSL 4.1, <http://fsl.fmrib.ox.ac.uk/fsl>) were used to obtain the normalized brain volume (NBV) and hippocampal volumes from the 3D FFE images. The scaling factor calculated with SIENAX was applied to obtain a normalized hippocampal volume (NHV). Before volume calculation, to correct for misclassification of WM lesions, all pixels classified as GM, but laying neither in cortical GM nor in subcortical GM, were reassigned to WM [Chard et al., 2010].

RS fMRI Data Pre-processing

To correct for minor head movements, SPM8 (www.fil.ion.ucl.ac.uk/spm) was used to realign the raw RS fMRI images to the mean of each session with a six degree rigid-body transformation. Data were subsequently normalized to the standard SPM8 EPI template using a non-linear transformation. Then, using the REST software (<http://resting-fmri.sourceforge.net>), a band-pass filtering between 0.01 and 0.08 Hz was applied to partially remove low-frequency drifts and physiological high-frequency noise, and subsequently to remove non-neuronal sources of synchrony between RS fMRI time series by regressing out the six motion parameters estimated by SPM8 and the average signals of the ventricular cerebrospinal fluid (CSF) and WM. Finally, images were smoothed using a 3D 6-mm Gaussian kernel.

RS FC Analysis

Statistical maps of RS FC between the left (L) and right (R) hippocampi, separately, and the remaining voxels of the brain were obtained for each subject using a seed-region correlation approach [Biswal et al., 1995]. Briefly, masks of the L and R hippocampi obtained for each subject using FIRST were coregistered and normalized to the RS fMRI mean image (obtained after realignment). Then, RS FC was investigated by calculating the correlation coefficients between the time series extracted from the L and R hippocampi and any other voxel in the brain. A Fisher's z transform was used to improve the Gaussianity of the obtained correlation coefficients [Lowe et al., 1998].

Statistical Analysis

Between-group differences in demographic and clinical variables were investigated using the χ^2 test and the Mann–

TABLE I. Main demographic, clinical and structural MRI findings from healthy controls and patients with multiple sclerosis (MS)

	HC	MS patients	P
Number of subjects	42	69	—
Women/men	22/20	36/33	0.6**
Mean age (SD) [years]	36.4 (11.1)	37.5 (9.4)	0.4*
Mean disease duration (range) [years]	—	10.8 (1–32)	—
Median EDSS (range)	—	1.5 (0.0–6.5)	—
Median MADRS score (range)	—	8.0 (0–30)	—
Mean T2 LV (SD) [mL]	—	6.7 (5.7)	—
Mean T1 LV (SD) [mL]	—	4.6 (4.4)	—
Mean NBV(SD) [mL]	1,584 (87)	1,522 (79)	<0.001*
Mean NHV (SD) [mL]	4.9 (0.5)	4.7 (0.5)	0.002*
Mean L NHV (SD) [mL]	5.0 (0.4)	4.6 (0.6)	<0.001*
Mean R NHV (SD) [mL]	4.9 (0.6)	4.7 (0.5)	0.7*

*Mann–Withney U test.

** χ^2 test.

HC = healthy controls; EDSS = Expanded Disability Status Scale; MADRS = Montgomery-Asberg Depression Rating Scale; LV = lesion volume; NBV = normalized brain volume; NHV = normalized hippocampal volume, R = right; L = left.

Whitney *U* test, as appropriate (SPSS software, version 21.0). Correlations between clinical and conventional MRI variables were assessed using the Spearman's Rank correlation coefficient.

Individual RS FC maps of *z*-scores were entered into SPM8 random-effect analysis to assess RS FC of L and R hippocampus, separately, in HC and patients with MS (one-sample *t* test), and between-group differences (two-sample *t* test, age and gender adjusted). In order to verify which clusters of between-group difference in RS FC were retained within the DMN, the comparison was repeated using the DMN template freely provided by GIFT software (<http://icatb.sourceforge.net/groupica.htm>) [Franco et al., 2009] as explicit mask. In patients with MS, multiple regression models were run to assess correlations of abnormal RS FC with clinical (disease duration, EDSS, MADRS) and structural MRI (T2 LV, T1 LV, NBV) variables. Correlations were investigated only in brain regions showing abnormal RS FC in patients with MS vs. HC, including age, gender, and NHV as nuisance covariates. All RS FC results were assessed at a threshold of $P < 0.05$, family-wise error corrected (FWE) for multiple comparisons and also tested at a $P < 0.001$, uncorrected (cluster extent = 20 voxels).

RESULTS

Demographic, Clinical, and Structural MRI Findings

The main demographic, clinical, and structural MRI characteristics of patients with MS and HC are reported in Table I. Results of the neuropsychological evaluation are reported in the Supporting Information Table. Thirty-nine patients (57%) were free from depressive symptoms (MADRS score = range 0–8), 27 patients (39%) had a mild

depression (MADRS score = range 9–17), and 3 patients (4%) had a moderate depression (MADRS score = range 18–34) [Mittmann et al., 1997]. Compared to HC, patients with MS had reduced NBV ($P < 0.001$), average NHV ($P = 0.002$), and left NHV ($P < 0.001$). No significant correlation was found between MADRS and EDSS score ($P = 0.4$), disease duration ($P = 0.6$), T2 LV ($P = 0.6$), T1 LV ($P = 0.8$), and NHV ($P = 0.7$). NHV was correlated with T2 LV ($r = -0.48$, $P < 0.001$) and EDSS ($r = -0.33$, $P < 0.005$).

Hippocampal RS FC

Areas showing positive significant correlations ($P < 0.001$, FWE corrected) with the L and R hippocampus, separately, in HC and patients with MS are shown in Figure 1. Both groups experienced a distributed RS FC of the L and R hippocampi with several limbic, neocortical, and basal ganglia regions. Compared with HC, patients with MS showed reduced RS FC between the L and R hippocampi and several regions located in the frontal, temporal and parietal lobes, the cingulate cortex, insulae, thalami, caudate nuclei, and cerebellum (Table II; Fig. 1). The large majority of clusters showing significantly decreased RS FC in patients with MS vs HC was located within the DMN (Table II). No area of increased hippocampal RS FC was found in patients with MS compared to HC.

Correlations Between Hippocampal RS FC and Structural MRI Measures

Table III and Figure 2 summarize the results of the analysis of correlation between hippocampal RS FC and structural MRI variables in patients with MS ($P < 0.001$). Higher T2 LV and T1 LV were significantly correlated to reduced hippocampal RS FC (both for the L and R hippocampus, r

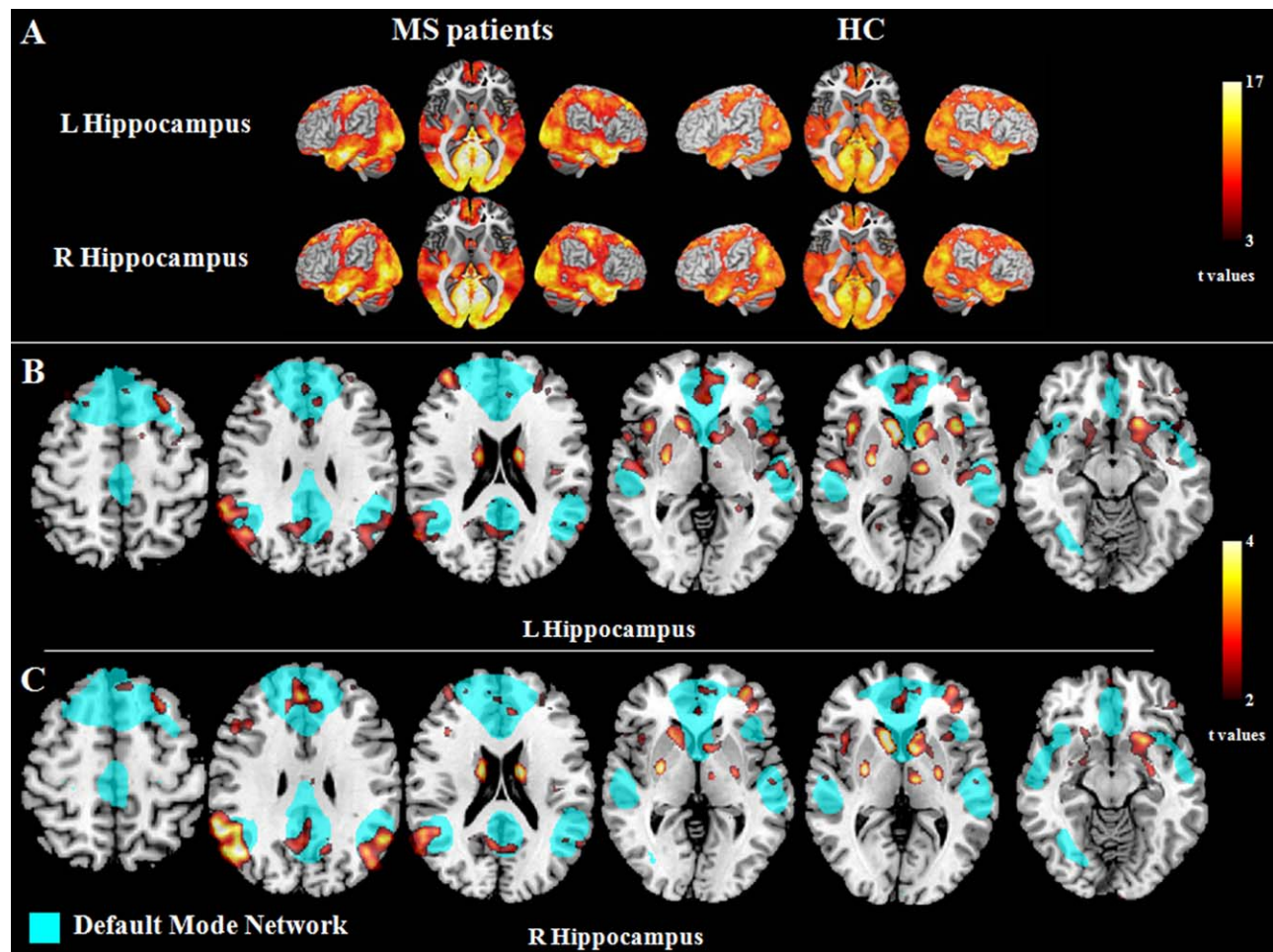


Figure 1.

Hippocampal RS FC in MS patients and HC. **A:** RS FC analysis showing the positive correlations between the left and right hippocampus and all other brain voxels in patients with MS and healthy controls (HC) (within-group analysis, one-sample t-tests, $P < 0.001$, family-wise error corrected). **B, C:** Areas of

decreased hippocampal RS FC in MS patients vs HC ($P < 0.001$, uncorrected) are superimposed onto a standard template mask of the default mode network (light blue areas) (Franco et al., 2009). Images are in radiological convention. See text for further details.

ranging from -0.33 to -0.46) with several areas located in the frontal-parietal-temporo-occipital lobes, bilaterally, corresponding to regions which are part of the DMN. Lower NBV was significantly related to reduced R hippocampal RS FC with the R superior frontal gyrus ($r = 0.36$) and with the R calcarine sulcus ($r = 0.39$).

Correlations Between Hippocampal RS FC and Depression

Table IV and Figure 3 summarize the results of the analysis of correlation between reduced hippocampal RS FC and depression in patients with MS ($P < 0.001$). Both for the L and R hippocampus, reduced RS FC with the R middle temporal gyrus was significantly correlated with the

severity of depression ($r = -0.35$ and -0.29 , respectively). For the R hippocampus, significant correlation was also found between higher MADRS and reduced RS FC with the L precuneus and R superior orbitofrontal gyrus ($r = -0.29$ and -0.32 , respectively).

Correlations Between Hippocampal RS FC and Other Clinical Measures

Table IV and Figure 3 summarize the results of the analysis of correlation between reduced hippocampal RS FC and clinical variables in patients with MS ($P < 0.001$). A longer disease duration was significantly correlated to reduced hippocampal RS FC (both for the L and the R hippocampus) with the contralateral hippocampus, L angular

TABLE II. Brain areas with reduced hippocampal resting state (RS) functional connectivity (FC) in multiple sclerosis (MS) patients compared to healthy controls (HC) ($p < 0.001$, uncorrected)

	Anatomical regions	Side	MNI coordinates			BA	t values	k	MNI coordinates (DMNI masked)			t values	k
			x	y	z				x	y	z		
Left hippocampus	Caudate nucleus [§]	R	8	10	10	—	4.99*	311	14	18	2	3.53	25
	Thalamus		16	-20	14	—	2.96	311	16	-20	14	3.0	5
	Caudate nucleus [§]	L	-8	10	10	—	4.88*	266	-14	20	6	3.0	10
	Thalamus		-10	-14	6	—	3.84	266	-10	-16	10	2.96	4
	Putamen [§]	R	26	-8	6	—	4.19*	56	18	18	-2	3.3	5
	Middle frontal gyrus [§]	L	-30	44	8	47	3.9*	25	-30	44	8	3.9	25
	Insula [§]	L	-34	18	6	48	3.9	49	-34	20	10	3.41	7
	Insula [§]	R	40	16	6	48	3.83	43	40	18	8	3.52	6
	Middle frontal gyrus	L	-24	24	56	8	3.12	10	—	—	—	—	—
	Supramarginal gyrus [§]	R	62	-46	32	48	3.74	51	62	-46	32	3.74	49
	Angular gyrus [§]	L	-58	-56	26	22	3.71	15	-58	-56	26	3.71	15
	Parahippocampal gyrus [§]	L	-30	-42	-6	37	3.58	10	-30	-42	-6	3.58	8
	Precuneus [§]	R	8	-60	24	23	3.35	10	8	-60	24	3.27	5
	Medial orbitofrontal gyrus [§]	R	2	58	-6	10	3.27	5	2	58	-6	3.20	4
	Superior temporal gyrus	L	-54	-14	2	48	3.2	5	—	—	—	—	—
Right Hippocampus	Cerebellum [§]	R	14	-44	-16	30	3.18	5	14	-44	-16	3.18	3
	Caudate nucleus [§]	R	10	16	10	—	5.37*	270	12	22	6	3.28	7
	Caudate nucleus [§]	L	-8	10	8	—	4.43	311	-12	20	6	3.2	5
	Thalamus		-10	-12	10	—	3.85	194	-10	-16	10	3.1	4
	Angular gyrus [§]	R	46	-60	38	39	4.35*	364	46	-60	38	4.35	362*
	Middle frontal gyrus [§]	L	-28	44	10	47	3.87	15	-28	44	10	3.87	15
	Angular gyrus [§]	L	-56	-56	26	22	3.79	21	-56	-56	26	3.79	21
	Putamen [§]	R	26	-6	8	—	3.72	37	20	20	-2	3.2	4
	Inferior frontal Gyrus [§]	R	38	24	26	48	3.42	12	38	24	26	3.42	7
	Putamen	L	-26	-8	6	—	3.33	8	—	—	—	—	—
	Cerebellum [§]	R	16	-26	-32	—	3.23	5	14	-44	-16	3.32	4
	Precuneus [§]	R	6	-60	24	23	3.12	5	6	-60	24	3.0	4
	Cingulum [§]	R	4	42	30	32	3.1	5	4	42	30	3.1	4

MNI = Montreal Neurological Institute; BA = Brodmann area; k = cluster extent.

* $p < 0.05$, family-wise corrected for multiple comparisons.

Clusters retained within the mask of the default mode network (DMNI) (Franco et al., 2009) are marked with[§], and corresponding cluster peaks and t values are provided

TABLE III. Correlations between structural MRI measures and resting state (RS) functional connectivity (FC) between the hippocampus (left and right) and other brain regions in multiple sclerosis (MS) patients ($P < 0.001$)

		Anatomical regions	Side	BA	MNI coordinates			t values ^a	r
					x	Y	z		
T2 LV	L Hippocampus	Superior frontal gyrus	R	10	18	64	2	2.7	-0.37
		Middle occipital gyrus	L	39	-40	-68	24	3.4	-0.44
		Precuneus	L	23	-10	-58	20	2.9	-0.39
	R Hippocampus	Precuneus	R	30	4	-60	18	3.9	-0.43
		Medial orbitofrontal gyrus	L	11	2	62	-8	3.9	-0.45
		Middle occipital gyrus	L	39	-42	-68	24	2.9	-0.36
T1 LV	L Hippocampus	Medial orbitofrontal gyrus	R	10	8	58	28	2.8	-0.34
		Precuneus	L	23	-10	-58	20	2.9	-0.39
		Middle temporal gyrus	L	39	58	-70	20	2.8	-0.37
		Posterior cingulum	R	23	2	-62	20	3.3	-0.43
	R Hippocampus	Middle occipital gyrus	L	39	-40	-68	24	3.0	-0.40
		Precuneus	L	23	-6	-64	20	3.7	-0.46
		Medial orbitofrontal gyrus	R	11	2	62	-8	3.6	-0.43
		Superior frontal gyrus	R	10	8	58	28	2.9	-0.35
		Middle occipital gyrus	L	39	-42	-68	24	2.8	-0.34
		Middle temporal gyrus	L	39	-46	-66	26	2.7	-0.33
NBV	R Hippocampus	Calcarine/precuneus	R	23	2	-62	18	3.0	0.39
		Superior frontal gyrus	R	10	22	70	8	2.8	0.36

^at values derived from linear contrasts on beta estimates of SPM8 multiple regression models; critical t value of significance=2.7.

MNI = Montreal Neurological Institute; BA = Brodmann area; LV = lesion volume; R = right; L = left; NBV = normalized brain volume.

Correlations were investigated only in brain regions showing abnormal RS FC in MS patients vs healthy controls.

gyrus, and L middle frontal gyrus (r ranging from -0.37 to -0.43). A longer disease duration was also significantly correlated with reduced RS FC between the L hippocampus and the R angular gyrus and middle temporal gyrus (MTG) ($r = -0.44$ and -0.39 , respectively), as well as between the R hippocampus, the R superior frontal gyrus, and the L middle cingulate cortex ($r = -0.34$ and -0.37 , respectively).

Higher EDSS was significantly correlated to reduced hippocampal RS FC (both for the L and R hippocampus) with the bilateral middle occipital gyrus, bilateral angular gyrus, and the L middle frontal gyrus (r ranging from -0.29 to -0.50).

DISCUSSION

In this study, we tested the hypothesis that clinical symptoms attributable to a hippocampal dysfunction in patients with MS might be, at least in part, due to a functional disconnection of this structure due to the presence of lesions in the brain WM. Previous RS FC studies in healthy subjects have demonstrated that the hippocampus is part of the DMN [Greicius et al., 2004; Vincent et al., 2006], an integrated brain system which is active at rest and plays an established role in the hierarchical organization of all brain networks [Crossley et al., 2014; Gusnard et al., 2001; Raichle et al., 2001]. Abnormalities of DMN RS FC are thought to be involved in the clinical symptomatology of a number of brain disorders, including MS

[Buckner et al., 2008]. Disrupted DMN connectivity has been also found in individuals with major depression [Bluhm et al., 2009; Chen et al., 2015; Zeng et al., 2012; Zhu et al., 2012]. As a consequence, among the many hippocampal RS FC links found to be damaged in patients with cognitively-intact MS, the disruption of those connecting the hippocampus to the main hubs of the DMN (i.e., the anterior medial prefrontal regions and the precuneus) [Buckner et al., 2008; Fox et al., 2005] are of particular interest in the context of depression. Such findings indicate the occurrence of an early detachment of the hippocampal functional subset from the rest of the DMN in patients with MS, independently from the development of relevant cognitive impairment, and suggest a link between altered DMN RS FC and the complex cognitive and emotional disturbances related to depression. Although additional longitudinal investigations in patients with MS with different degrees of cognitive deficits are needed to assess the impact of such a hippocampal disconnectivity on behavioral and cognitive functions, it is worth noting that in our cohort hippocampal disconnection was correlated to disease duration, suggesting a loosening of hippocampal functional synchronization with time in these patients.

The notion that the hippocampus is involved in the pathogenesis of depression is supported by previous studies in psychiatric patients with major depression [Koolschijn et al., 2009] and depressed MS patients [Gold et al., 2014; Kiy et al., 2011], which have shown that tissue loss in this structure is related to the severity of depression.

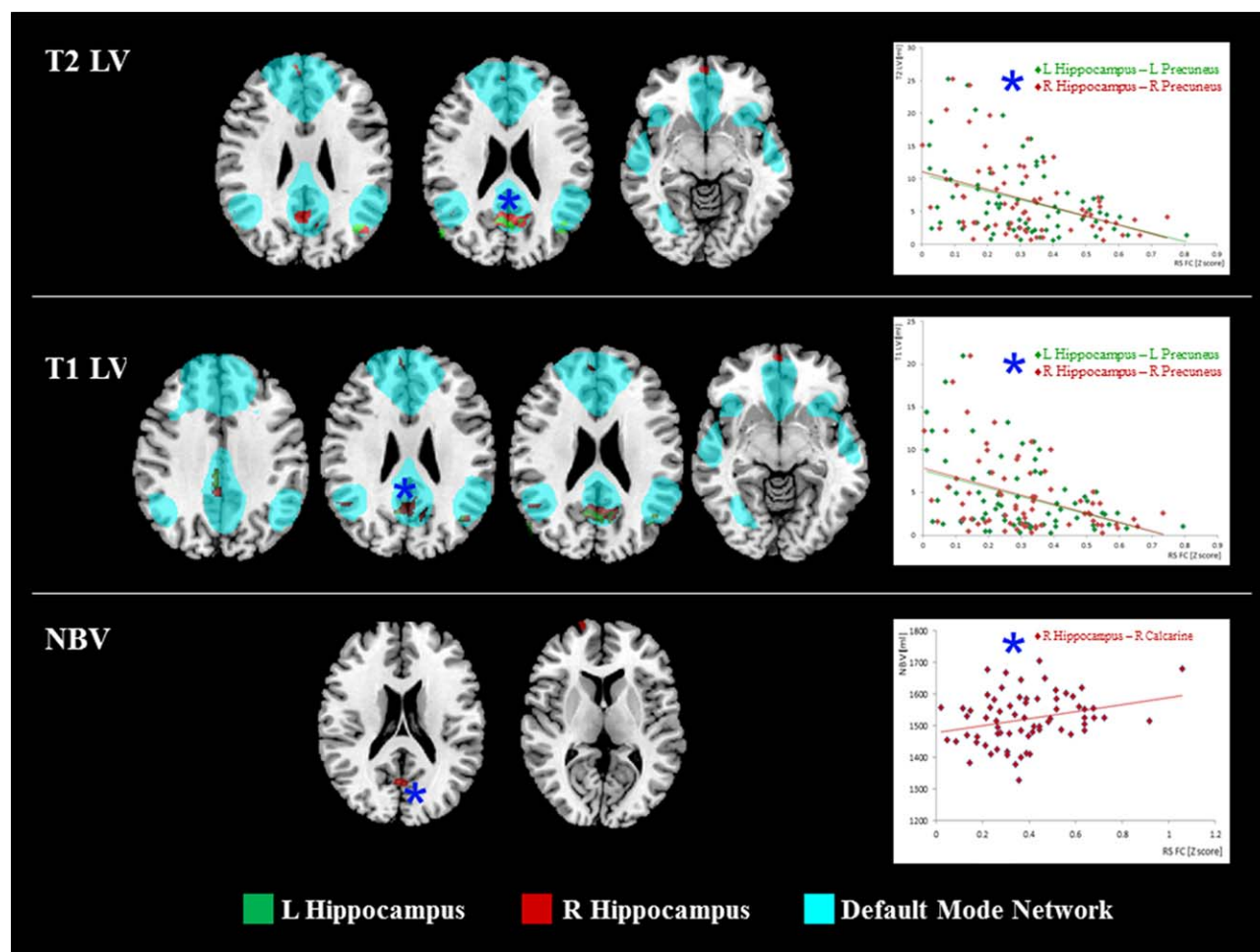


Figure 2.

Correlation between hippocampal RS FC and MRI measures in MS patients. Results (and illustrative scatterplots) of the analysis of correlation in patients with MS between reduced RS FC of the left (L) (green) and right (R) (red) hippocampus and brain T2 lesion volume (LV), T1 LV, and normalized brain volume (NBV) ($P < 0.001$, uncorrected). The T2 LV and T1 LV results

are superimposed onto a standard template mask of the default mode network (light blue areas) (Franco et al., 2009). Green scatterplots refer to correlations with L hippocampal RS FC, while red scatterplots refer to correlations with R hippocampal RS FC. Images are in radiological convention. See text for further details.

Our study showed a significant association between depression and reduced RS FC in the orbitofrontal cortex, MTG, and precuneus. The limbic-cortical circuit involving the medial orbitofrontal cortex is considered important in mood and affect regulation [Mayberg, 2003], and the involvement of orbitofrontal regions in depression has been well documented, both in patients with MS [Feinstein et al., 2004; Gobbi et al., 2013] and in other neurological conditions [Canu et al., 2015; Kostic et al., 2010; Mayberg et al., 1992]. The MTG is located in the dorsal attention system and is involved in cued attention and working memory. The correlation we found between decreased MTG connectivity and higher MADRS scores is in line with previous studies of major depression [Ma et al., 2012]

and might be related with an abnormal processing of complex emotional visual stimuli. The precuneus has a crucial role in the integration of mental processing, through its role in cognitive control processes such as visual imagery, episodic memory, and self-directed operations [Cavanna and Trimble, 2006]. Abnormal RS FC of the precuneus has been found in depressed patients [Peng et al., 2015; Zhu et al., 2012], which was thought to be linked to a significant dissociation between the DMN and relevant sensory, motor, and attention-processing systems. In patients with MS, a more prominent role for depression of the right hippocampus, particularly in women, was postulated by structural MRI investigations [Gold et al., 2014]. In our cohort (which included both women and

TABLE IV. Correlations between clinical measures and resting state (RS) functional connectivity (FC) between the hippocampus (left and right) and other brain regions in multiple sclerosis (MS) patients ($P < 0.001$)

		Anatomical regions	Side	BA	MNI coordinates			t values ^a	r	
					x	y	z			
MADRS	L Hippocampus	Middle temporal gyrus	R	22	66	-48	12	3.0	-0.35	
	R Hippocampus	middle temporal gyrus	R	21	64	-50	16	2.7	-0.29	
		Precuneus	R	7	10	-64	38	2.8	-0.29	
Disease duration		Superior orbitofrontal gyrus	L	11	-18	48	-12	2.8	-0.32	
	L Hippocampus	Hippocampus	L	27	-20	-38	4	3.2	-0.42	
		Angular gyrus	L	39	-54	-52	36	2.9	-0.37	
		Angular gyrus	R	39	54	-66	32	3.4	-0.44	
		Middle frontal gyrus	L	9	-30	30	44	3.2	-0.42	
		Middle temporal gyrus	R	22	54	-50	18	3.1	-0.41	
		R Hippocampus	Angular gyrus	R	39	42	-64	36	3.0	-0.39
		Angular gyrus	L	39	-42	-66	44	3.2	-0.41	
		Middle frontal gyrus	L	9	-30	28	44	3.3	-0.43	
		Superior frontal gyrus	R	10	10	56	28	2.7	-0.34	
		Middle cingulate cortex	L	23	-2	-36	42	2.8	-0.37	
		L Hippocampus	Middle occipital gyrus	R	39	40	-80	24	4.4	-0.50
EDSS		Middle frontal gyrus	L	46	-40	34	40	2.7	-0.31	
		Middle occipital gyrus	L	39	-46	84	28	4.1	-0.48	
		Precuneus	L	7	-6	-66	50	2.7	-0.32	
		R Hippocampus	Middle occipital gyrus	L	39	-58	-70	24	3.8	-0.44
		Middle frontal gyrus	L	45	-42	32	38	2.7	-0.33	
		Angular gyrus	R	39	36	-66	42	3.2	-0.39	
		Middle occipital gyrus	R	39	44	-78	28	2.7	-0.29	
		Angular gyrus	L	7	-36	-60	30	3.1	-0.38	

^at values derived from linear contrasts on beta estimates of SPM8 multiple regression models; critical t value of significance=2.7.

MNI = Montreal Neurological Institute; BA = Brodmann area; L = left; R = right; EDSS = Expanded Disability Status Scale; MADRS = Montgomery-Asberg Depression Rating Scale.

Correlations were investigated only in brain regions showing abnormal RS FC in MS patients vs healthy controls.

men), a correlation between abnormalities of RS FC and depression was found for both the right and left hippocampus, thus not supporting such a laterality effect. Noteworthy, abnormalities of hippocampal connectivity do not seem to be driven by atrophy of this structure, since tissue loss was present only in the left and not the right hippocampus, suggesting that modifications of function might precede those of structure in the pathogenesis of depression. Clearly, as suggested by post mortem investigations, we cannot exclude that the presence of other pathological abnormalities in the hippocampi, including focal lesions and extensive demyelination [Dutta et al., 2011; Geurts et al., 2007], might have led to such an hippocampal dysfunction. In this perspective, synaptic alterations have been correlated to hippocampal demyelination in the absence of neuronal loss in a post mortem study [Dutta et al., 2011].

Differently from previous atrophy studies, which found no relationship between hippocampal abnormalities and disability [Longoni et al., 2015; Sicotte et al., 2008], in our study hippocampal RS FC disruption was correlated to the severity of disability, which might be due to the role played by the hippocampus in the control of the so-called ventral striatal loop, a complex circuit including the fornix,

ventral striatum, ventral pallidum, and prefrontal cortex, which is involved in motor behavior [Duvernoy, 2005]. We tend to exclude that the correlation we found between RS FC abnormalities and disability might be influenced by depression, since no significant association was found between EDSS and MADRS, and only a minimal overlap was found between regions of abnormal RS FC correlated with EDSS and those correlated with MADRS.

Another intriguing finding of our investigation comes from the analysis of correlation between hippocampal RS FC abnormalities and lesional measures of disease burden in the WM, which showed a distributed association between reduced hippocampal RS FC with many DMN regions and higher T2 LV and T1 LV. These results suggest that the accumulation of focal WM lesions diffusely in the brain is likely to be one of the factors affecting the altered interaction between the hippocampal formation and other regions of the DMN. The notion that WM lesions have an influence on the hippocampus in MS is not novel, since it has been shown by previous atrophy studies, which found more pronounced hippocampal atrophy in patients with higher T2 LV [Longoni et al., 2015; Sicotte et al., 2008]. We found that such an effect of lesions on the hippocampus might also result in a disturbed

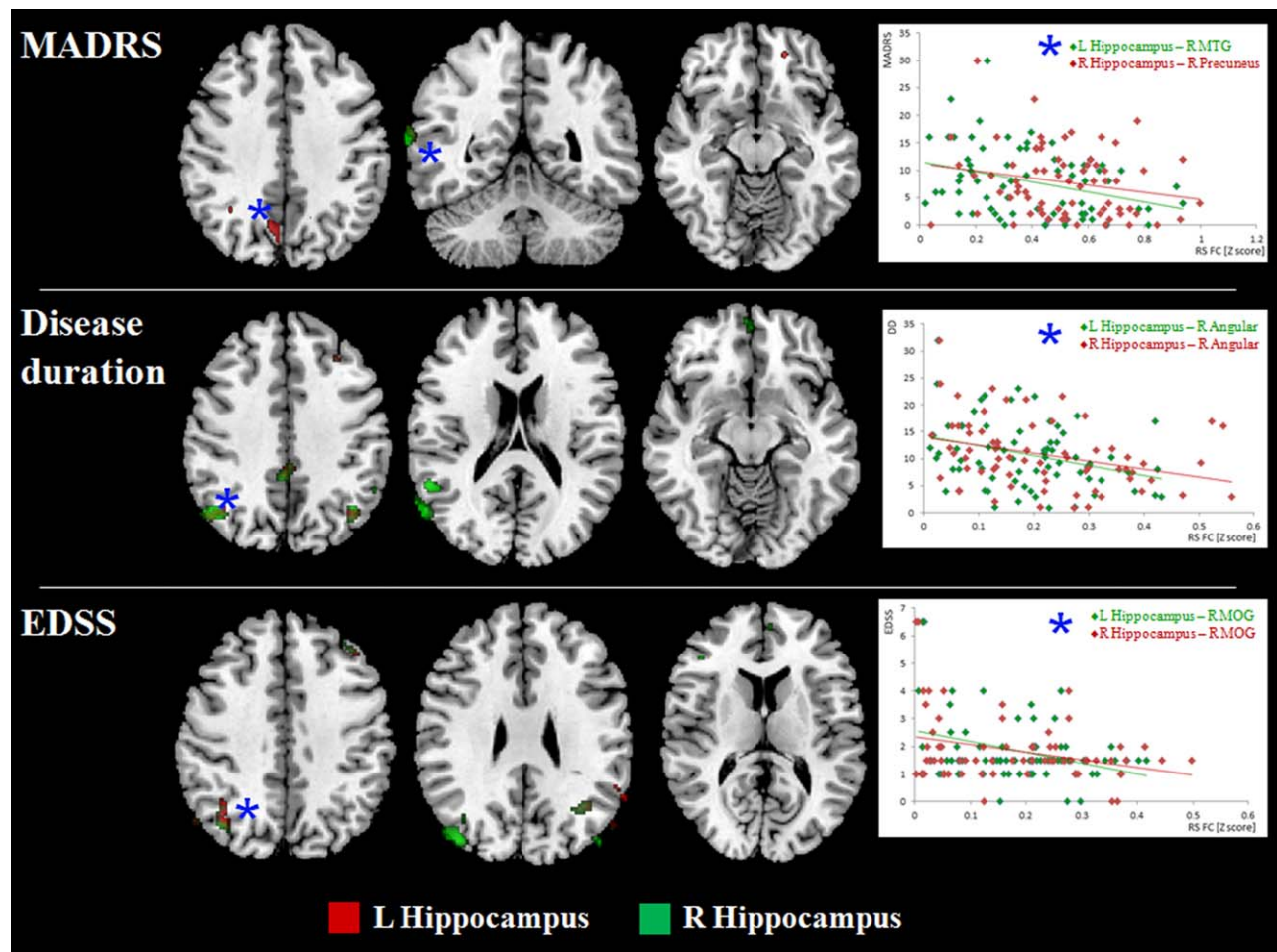


Figure 3.

Correlation between hippocampal RS FC and depression and clinical measures in MS patients. Results (and illustrative scatterplots) of the analysis of correlation in patients with MS between reduced RS FC of the left (L) (green) and right (R) (red) hippocampus and Montgomery–Asberg Depression Rating Scale (MADRS), disease duration, and Expanded Disability Status Scale

(EDSS) ($P < 0.001$, uncorrected). Green scatterplots refer to correlations with L hippocampal RS FC, while red scatterplots refer to correlations with R hippocampal RS FC. Images are in radiological convention. See text for further details. Abbreviations: MTG = middle temporal gyrus; MOG = middle occipital gyrus.

functional interaction at a network level. Different mechanisms might contribute to explain the effect of lesions on hippocampal RS FC, including transynaptic degeneration of afferent/efferent WM tracts connected to the hippocampus passing through WM lesions and/or the selective involvement of WM tracts which contribute to the architecture of the DMN (e.g., the cingulum, superior-fronto-occipital fasciculus, and corpus callosum) [van den Heuvel et al., 2009]. The effect of lesions on hippocampal disconnection was much more pronounced and distributed than that of global atrophy, which influenced hippocampal connectivity only with a few and sparse cortical areas. This suggests that WM macroscopic damage accumulation and tissue loss play

different roles in the pathophysiology of hippocampal dysfunctional changes.

Our study is not without limitations. First, it is cross-sectional. As a consequence it was not possible to establish the exact temporal relationship between WM lesion formation and hippocampal disconnection, and clinical outcomes, including depression. Future longitudinal investigations are therefore warranted. Second, correlations between RS FC abnormalities and structural damage were assessed by using T2/T1 LV, which are global, relatively unspecific measures of brain damage. Future studies including more sophisticated measures of structural damage (such as diffusion tensor MRI and tractography) might be able to disclose closer associations between depression,

RS FC abnormalities, and regional distribution of WM abnormalities. Third, hippocampal function was investigated by analyzing depression. As a consequence, these findings need to be replicated for memory impairment, another deficit related to hippocampal damage/dysfunction. However, the selection of patients with MS with isolated memory impairment might result relatively difficult, since these patients typically experience a multidomain cognitive impairment. Finally, the evaluation of hippocampal structural and functional circuitry in patients at the clinical onset of the disease (i.e., clinically isolated syndromes) is of paramount importance to define whether such a hippocampal disconnectivity might represent an early biomarker of the disease, useful to monitor the dynamics of its manifestations and progression.

AUTHORS CONTRIBUTION

M.A. Rocca: study concept and design; acquisition of data; analysis and interpretation of data; drafting the article, and final approval of the version to be published. E. Pravata: analysis and interpretation of data; drafting the article, and final approval of the version to be published. P. Valsasina: study design; statistical analysis; analysis and interpretation of data; revising the article for important intellectual content; and final approval of the version to be published. M. Radaelli: patient recruitment; revising the article for important intellectual content; and final approval of the version to be published. B. Colombo: patient recruitment; revising the article for important intellectual content; and final approval of the version to be published. L. Vacchi: neuropsychological evaluation (including assessment of fatigue and depression), analysis and interpretation of data; revising the article for important intellectual content; and final approval of the version to be published. C. Gobbi: interpretation of the data; revising the article for important intellectual content; and final approval of the version to be published. G. Comi: interpretation of the data; revising the article for important intellectual content; and final approval of the version to be published. A. Falini: acquisition of the data; revising the article for important intellectual content; and final approval of the version to be published. M. Filippi: study concept and design; revising the article for important intellectual content; and final approval of the version to be published; study supervision and coordination.

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