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ORIGINAL RESEARCH

Hippocampal structural and functional integrity in multiple sclerosis patients with or without memory impairment: a multimodal neuroimaging study

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Abstract
The increasing evidence for a pure amnestic-like profile in multiple sclerosis (MS) introduces the role of hippocampal formation in MS episodic memory function. The aim of the present study was to investigate structural and functional hippocampal changes in mildly-disabled MS patients with and without memory impairment. Thirty-one MS patients with or without memory impairment and 16 healthy controls (HC) underwent MRI in a 3.0 T MRI scanner. Patients were categorized as memory preserved (MP) and memory impaired (MI) based on verbal and visual memory scores extracted from the Brief Repeatable Neuropsychological Battery. The acquisition protocol included high-resolution 3D-T1-weighted, diffusion weighted imaging and echo-planar imaging sequences for the analysis of hippocampal gray matter (GM) density, perforant pathway area (PPA) tractography, and hippocampal functional connectivity (FC), respectively. Compared to HC, we found decreased left and bilateral hippocampal GM density in MP and MI patients, respectively, decreased fractional anisotropy and increased radial diffusivity on left PPA in MI patients, and reduced FC in MI between left hippocampus and left superior frontal gyrus, precuneus/posterior cingulated cortex and lateral occipital gyrus/angular gyrus. The only differences between MP and MI were found in FC. Specifically, MP patients showed FC changes between left hippocampus and right temporo-occipital fusiform/lingual gyrus (increased FC) as well as supramarginal gyrus (decreased FC). In conclusion, we highlight the early detection of structural hippocampal changes in MS without neuropsychologically-detected memory deficits and decreased hippocampal FC in MS patients with impaired memory performance, when both GM density and PPA integrity are affected.

Keywords Hippocampus · Voxel based morphometry · Perforant pathway tractography · Resting state functional connectivity · Episodic memory

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Introduction

Despite the fact that information processing speed and working memory constitute the hallmarks of impaired cognitive profile in multiple sclerosis (MS) patients, episodic memory has also been found to be commonly affected (Thornton and Raz 1997), even in patients with clinically isolated syndrome (CIS) (Feuillet et al. 2007) or in definite MS cases without significant disability, early in the course of the disease (Deloire et al. 2010). MS-related memory impairment has been associated with reduced processing speed (Brissart et al. 2012) and the underlying subcortical white matter (WM) pathology (Dineen et al. 2009). However, increasing evidence for a pure amnestic-like profile (Thornton and Raz 1997) introduces the role of medial temporal lobe and hippocampal formation in MS episodic memory function.

Both histopathological and in vivo neuroimaging studies have found hippocampal demyelination (Geurts et al. 2007; Papadopoulos et al. 2009), focal hyperintensities (Roosendaal et al. 2008), grey matter (GM) atrophy (Audoin et al. 2010; Sicotte et al. 2008), gliosis (Geurts et al. 2006) and reduced glucose metabolism (Paulesu et al. 1996) in MS patients. But it was until recently in the long history of MS-related cognitive impairment that the introduction of advanced neuroimaging techniques with semi-automated and automated post-processing methods highlighted the predictive validity of measurable hippocampal changes in episodic memory (Benedict et al. 2009).

In early disease stages, a dissociation between the neuroanatomical (i.e. structural and/or functional) and behavioral (i.e. cognitive) changes has been detected. Roosendaal et al. (2010) reported significant reduced right hippocampal volume in MS patients with intact spatial memory which was accompanied by decreased resting-state functional connectivity (FC) between hippocampus and several cortical areas. Furthermore, the microstructural integrity of hippocampus (measured via diffusion tensor imaging [DTI] and measures of fractional anisotropy [FA] and mean diffusivity [MD]) have been reported to be early compromised and discriminate CIS patients with and without memory impairment (Planche et al. 2017a, b, c). In addition, MS patients with preserved memory function show increased FC within the default-mode network (DMN) as compared with memory-impaired patients. DMN includes limbic structures associated with memory function, i.e. hippocampus, anterior cingulate cortex (ACC), posterior cingulate cortex (PCC) (Greicius et al. 2003) and is particularly involved (reduced FC) in patients with pronounced memory deficits (Bai et al. 2008; Rombouts et al. 2005; Sorg et al. 2007; Wang et al. 2006). The increased FC within the DMN in patients with intact memory function highlights a possible compensatory process in early disease stage. On the other hand, increased FC between hippocampus and several cortical regions has been recently detected in association to the severity of memory impairment; patients without memory impairment present increased FC which is further increased in patients with neuropsychologically detected memory impairment (González Torre et al. 2017). To our knowledge, a multimodal approach that simultaneously combines structural measures of hippocampal GM volume and WM integrity of the hippocampal bundle that is specific to episodic memory processes (i.e. perforant pathway area [PPA]), as well as functional measures of hippocampal connectivity with other brain regions is lacking.

Thus, the aim of the present study was to study structural and functional hippocampal changes in mildly disabled MS patients with and without memory impairment as defined with verbal and visual episodic memory measures.

Material and methods

Participants

Thirty one patients with demyelinating disease with or without memory impairment and 16 age-, gender-demographically-matched healthy controls (HC) were included in the study. In demyelinating disease group, 16 participants were diagnosed with clinically isolated syndrome (CIS) and 15 with relapsing-remitting MS (RRMS) (Polman et al. 2011). Inclusion criteria were: (1) age $\geq$ 18 years old, (2) Greek as native language, (3) formal education ($\geq$ 6 years); right-handedness. Exclusion criteria were: (1) absence of neurologic diseases (other than MS), (2) severe psychiatric disorders (including current major depressive symptoms), (3) absence of corticosteroid treatment and relapses the last 3 months, (4) absence of alcohol abuse or psychoactive drugs, (5) absence of developmental disorders (e.g. dyslexia), (6) Mini-Mental State Examination score > 23 (Fountoulakis et al. 2000), (7) Hospital Anxiety and Depression Scale <8 (Michopoulos et al. 2008). For patients, disease duration was recorded in years and clinical disability was measured using the Expanded Disability Status Scale (EDSS; Kurtzke 1983. All the participants provided informed consent for the study which was approved by local ethical committee.

Cognitive examination

Cognitive assessment was performed in patients using the Brief Repeatable Neuropsychological Battery (BRBN) (Boringa et al. 2001; Rao et al. 1991) adapted in the Greek language. Based on normative data (Potagas et al. 2008), patients were categorized with preserved or impaired memory performance (MP and MI, respectively) according to their scores on delayed recall on Bushke Selective Reminding Test (SRT) and 10/36 Spatial Recall Test (SPART) using the 5th percentile as a cut-off score. Patients were considered as MI with impaired performance either in SRT or SPART delayed recall. Patients categorized as MP did not show impaired performance (cut-off: 5th percentile) neither in delayed recall of SRT or SPART nor in any other episodic memory indices of SRT (sum of words recalled through learning trials, long-term storage, consistent long-term retrieval) or SPART (sum of items recalled through learning trials).

MRI data acquisition

All participants underwent the same imaging protocol on a 3 T Achieva TX Philips manufactured MRI scanner (Philips, Best, the Netherlands). The protocol included a 3D high resolution T1 (3D-HR-T1) weighted sequence (repetition time (TR): 9.9 ms, time echo (TE): 3.7 ms, flip angle: 7^0, voxel size 1x1x1 mm, sagittal orientation), DTI sequence (TR: 7299 ms, TE: 68 ms, flip angle: 90^o, acquisition voxel size: $2 \times 2 \times 2$ mm, sensitivity encoding reduction factor of 2, two b factors with 0 s/mm^2 [low b] and 1.000 s/mm^2 [high b]), T2* weighted gradient echo combined with echo planar imaging for resting state functional magnetic resonance imaging (rs-fMRI) (TR: 2000 ms, TE: 30 ms, flip angle:90^o, acquisition voxel size 3x3x3 mm and sensitivity encoding reduction factor of two), as well as T2 weighted fluid attenuated inversion recovery (T2-FLAIR) sequence. During the rs-fMRI scan participants were instructed to lie still with their eyes closed. An experienced neuroradiologist considered major anatomical abnormalities on participants' T1-weighted and T2-FLAIR images of the whole brain and also calculated the total lesion load using lesion growth algorithm (Schmidt et al. 2012) as implemented in the "LST: lesion segmentation toolbox" v. 2.0.15 (www.statisticalmodelling.de/lst.html) for SPM. Also, all data were checked slice by slice by an experienced MR physicist to indentify motion or other type of artifacts.

MRI data analysis

GM analysis: voxel-based morphometry (VBM)

Anatomical 3D-HR-T1 weighted images were analyzed with the computational anatomy toolbox (CAT12) of Statistical Parametric Mapping (SPM12; Wellcome Department of Cognitive Neurology, www.fil.ion.ucl.ac.uk/spm/software/spm12) using MATLAB R2015b (The MathWorks, Natick, USA). We applied the standard procedure implemented in CAT12. All images were normalized in Montreal Neurological Institute (MNI) space using the default International Consortium for Brain Mapping template and segmented into GM, WM and cerebrospinal fluid (CSF). The normalised modulated GM images were smoothed with a 8 mm full-width-at-half-maximum (FWHM) isotropic Gaussian kernel and used for further ROI-based analysis. Left and right hippocampus was used in ROI analysis. Between-group differences on GM density were tested in the following contrasts for both directions: HC vs. MP; HC vs. MI; MP vs. MI. Age, gender and total intracranial volume (TIV) were used as nuisance variables. The threshold for the resultant statistic maps was set at $p < 0.05$, corrected for multiple comparisons with family-wise error (FWE) correction. We further used a more liberal threshold at $p < 0.005$ (uncorrected at voxel-level) and cluster-level of 20 contiguous voxels per cluster that also survived small-volume correction (SVC) using a sphere with a radius of 10 mm (resulted at $p < 0.05$ FWE at peak cluster-level).

WM data analysis: DTI tractography

For the purpose of current DTI analysis, DTI data were processed using the Brainance DTI Suite (Advantis Medical Imaging, Eindhoven, the Netherlands) and tractography of the PPA was conducted. All DTI data sets underwent motion and eddy-current correction with the scanner registration tool and the co-registration protocol included in Brainance DTI suite before further analysis. We applied ROI-based fiber tractography of the PPA using multiple ROI approach (Christidi et al. 2011, 2017). At a coronal plane the most posterior slice was selected at the level of the middle of the splenium under the corpus callosum and a region of the PPA under the corpus callosum was identified (ROI 1). The second slice was anterior to the first one, generally at the level of the brainstem and at the level where the dentate nucleus of the cerebellum was prominent (ROI 2). The third and most anterior ROI was placed anterior to the pons at the level where the projections of the posterior limb of the internal capsule were prominent and the ROI was incorporated the area of WM of the mesial temporal lobe just underlying the hippocampus (ROI 3). Mean FA, axial and radial diffusivity (AD and RD, respectively) were extracted and used as the quantitative DTI measures of the PPA for left and right hippocampus. Between-group comparisons were conducted with non-parametric statistics (at $p < 0.05$), using IBM SPSS Statistics v. 22.0. Considering the small sample size of the groups, we further evaluated the between-groups magnitude of differences (Cohen's |r| effect size for non-parametric comparisons; Cohen 1988).

Functional data analysis

The CONN-fMRI Functional Connectivity toolbox v15 (http://www.nitrc.org/projects/conn) was used to extract individual subject seed-to-voxel connectivity maps. All functional and anatomical 3D-HR-T1 weighted images were preprocessed following the standard procedure implemented in Conn toolbox (Whitfield-Gabrieli and Nieto-Castanon 2012). Linear regression and band-pass filtering (0.01–0.1 Hz) were applied to remove unwanted motion, physiological, and other artifacts from the blood oxygen-level dependent (BOLD) signal before computing connectivity measures. Predefined bilateral hippocampus ROIs were used as seed points to identify the corresponding FC networks. General linear model was performed to explore the association between the mean time series from each hippocampal seed ROI and each voxel of the whole brain. Regressors corresponding to the 6 motion correction parameters, and their first temporal derivatives, global WM, and CSF were also included to remove variance related to motion, global WM, and CSF signals, respectively. To explore between-group differences on bidirectional hippocampal FC we performed second-level analyses, setting peak voxel threshold at $p < 0.005$ uncorrected and cluster extent threshold of $p < 0.05$ FWE corrected, using age and gender as nuisance covariates.

Statistical analysis

Descriptive measures were obtained for all demographic, clinical and cognitive data. Between-group comparisons on demographic, clinical and cognitive data were conducted using t-test, non-parametric Mann-Whitney test, one-way analysis of variance and chi-square. The IBM SPSS v. 22.0 was used for all analyses and the level of significance was set at $p < 0.05$.

Results

Group demographic characteristics and cognitive measures

Table 1 presents demographic and clinical characteristics of all groups. We didn't find significant differences except from the education level. Table 2 presents MP and MI performance on cognitive measures, as well as the proportion of patients with impaired performance based on normative data. As expected, MI patients scored significantly worse on SRT and SPART measures. They also performed significantly worse on Symbol Digit Modalities Test (SDMT) and Word List Generation (WLG) measures. Based on the percentage of patients with impaired performance (cut-off: 5th percentile), we found significantly more impaired patients ($p < 0.05$) in MI group with regard to SRT-LTS, SRT-DR, SPART-DR, SDMT and WLG.

GM volumetric measures

We didn't detect significant differences at $p < 0.05$ FWE corrected, and thus we applied a more liberal threshold at $p < 0.005$ uncorrected (with cluster-extent threshold and SVC of a 10 mm sphere) (Fig. 1). We found decreased GM density in bilateral hippocampus in MI patients and in the right hippocampus in MP patients compared to HC. Comparison between MP and MI groups did not reveal any significant differences.

WM tractography analysis of PPA

We did not find significant differences between HC and MP in DTI indices of the left and right PPA. On the other hand, MI patients had significantly decreased FA ($p = 0.002$) and increased RD ($p = 0.020$) on left PPA compared to HC. We did not observe significant differences between MP and MI patients. We found medium ($0.10 \leq |r| \geq 0.30$) to large ($|r| \geq 0.50$) effect sizes between HC and MI patients in left FA ($|r| = 0.56$) and AD ($|r| = 0.43$), as well as right FA ($|r| = 0.34$). Additionally, medium effect size was found for the left FA between HC and MP patients ($|r| = 0.30$). PPA integrity results are presented on Table 3.

Functional connectivity

Seed region: left hippocampus

MP vs. HC Compared to HC, MP patients did not show significantly decreased or increased FC between left hippocampus and other brain regions ($p < 0.005$ uncorrected at voxel level; $p < 0.05$ FWE at cluster level).

MI vs. HC MI patients showed significantly decreased FC compared to HC between left hippocampus and left superior frontal

Table 1 Demographic and clinical characteristics of the groups of MP and MI patients and HC

	HC	MP	MI	*p*-value
Age	37.50 ± 7.38	37.37 ± 6.60	38.58 ± 7.75	*ns*
Gender (M/F)	6/10	5/14	2/10	*ns*
Education level	14.06 ± 1.65	15.32 ± 1.16	13.83 ± 2.17	$p = 0.026$
MS subtype (CIS/RRMS)	–	11/8	5/7	*ns*
Disease duration	–	9.75 ± 0.71	11.14 ± 3.93	*ns*
EDSS	–	1 ± 0.87	1.67 ± 1.21	*ns*
TIV	1453.91 ± 169.12	1674.37 ± 137.88	1474.50 ± 132.45	*ns*
TLL	–	9.63 ± 6.11	13.5 ± 6.79	*ns*

HC healthy controls, *MP* patients with preserved memory, *MI* patients with impaired memory, *M/F* male/female, *MS* multiple sclerosis, *CIS* clinically isolated syndrome, *RRMS* relapsing-remitting multiple sclerosis, *EDSS* Expanded Disability Status Scale, *TIV* total intracranial volume, *TLL* total lesion load, *ns* non-significant. With the exception of categorical variables (gender; MS subtype), all other variables are presented as mean (standard deviation)

Table 2 Cognitive performance of MP and MI patients on BRNB tests

BRNB tests	Cognitive scores (Mean ± SD)		Statistics MP vs. MI (p; Cohen's $\|r\|$)
	MP	MI	
SRT-CLTR	35.21 ± 10.07	23.42 ± 11.52	$p = 0.007$; $\|r\| = 0.47$
SRT-LTS	47.42 ± 8.15	37.42 ± 9.20	$p = 0.003$; $\|r\| = 0.52$
SRT-DR	8.42 ± 1.68	6.33 ± 2.06	$p = 0.007$; $\|r\| = 0.48$
SPART-Total	23.84 ± 3.82	19.92 ± 4.93	$p = 0.025$; $\|r\| = 0.40$
SPART-DR	9.32 ± 0.89	6.83 ± 2.21	$p = 0.004$; $\|r\| = 0.53$
SDMT	57.05 ± 8.55	49.00 ± 10.90	$p = 0.035$; $\|r\| = 0.38$
PASAT-3	48.16 ± 9.59	42.30 ± 11.49	$p = 0.164$ $\|r\| = 0.26$
PASAT-2	33.89 ± 10.23	32.33 ± 4.00	$p = 0.860$; $\|r\| = 0.04$
WLG	22.72 ± 4.72	18.09 ± 5.78	$p = 0.039$; $\|r\| = 0.37$

MP patients with preserved memory, *MI* patients with impaired memory, *BRNB* Brief Repeatable Neuropsychological Battery, *SRT* Selective Reminding Test, *CLTR* Consistent Long-Term Retrieval, *LTS* Long-Term Storage, *DR* Delayed Recall, *SPART* 10/36 Spatial Recall Test, *SDMT* Symbol Digit Modalities Test, *PASAT* Paced Auditory Serial Attention Test, *WLG* Word List Generation. Comparisons between MP and MI groups were conducted using Mann-Whitney test. Cohen's |r| effect size (Cohen 1988) corresponds to small (0.10), medium (0.30) and large (0.50)

gyrus, left precuneus/PCC and left lateral occipital gyrus/angular gyrus ($p < 0.005$ uncorrected at voxel level; $p < 0.05$ FWE at cluster level) (Fig. 2). There were not regions showing increased connectivity with left hippocampus in MI compared to HC.

MP vs. MI The comparison between MP and MI patients revealed increased FC in MP patients between left hippocampus and right temporo-occipital fusiform/lingual gyrus ($p < 0.005$ uncorrected at voxel level; $p < 0.05$ FWE at cluster level). MP

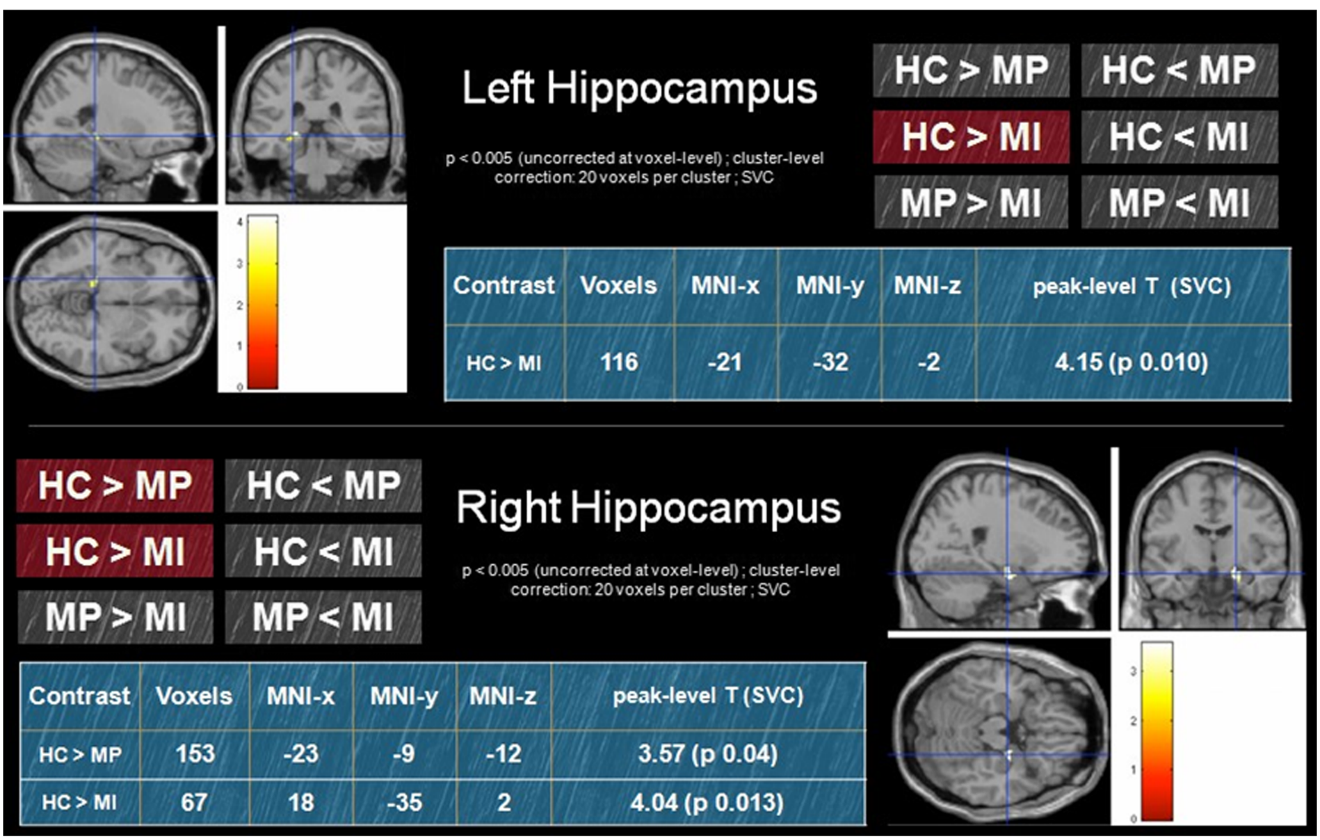

Fig. 1 Localization of the left and the right hippocampal ROI in 3-plane anatomical T1 image and between-group differences (HC vs. MP; HC vs. MI; MP vs. MI)

Table 3 Mean FA, AD and RD of the PPA for MP and MI patients and HC

DTI indices	Groups			Statistics (p; effect size $\|r\|$)		
	HC	MP	MI	HC vs MP	HC vs MI	MP vs MI
Left PPA						
FA	0.41 ± 0.02	0.40 ± 0.02	0.39 ± 0.01	$p = 0.106$; $\|r\| = 0.28$	$p = 0.002$; $\|r\| = 0.56$	$p = 0.120$; $\|r\| = 0.28$
AD	1.22 ± 0.04	1.21 ± 0.06	1.22 ± 0.04	$p = 0.483$; $\|r\| = 0.12$	$p = 0.781$; $\|r\| = 0.05$	$p = 0.617$; $\|r\| = 0.09$
RD	0.63 ± 0.03	0.65 ± 0.05	0.66 ± 0.02	$p = 0.255$; $\|r\| = 0.20$	$p = 0.020$; $\|r\| = 0.43$	$p = 0.535$; $\|r\| = 0.12$
Right PPA						
FA	0.42 ± 0.02	0.40 ± 0.02	0.40 ± 0.02	$p = 0.084$; $\|r\| = 0.30$	$p = 0.076$; $\|r\| = 0.34$	$p = 0.921$; $\|r\| = 0.02$
AD	1.20 ± 0.04	1.21 ± 0.06	1.20 ± 0.07	p 0.01; $\|r\| = 0.20$	$p = 0.980$; $\|r\| = 0.01$	$p = 0.984$; $\|r\| = 0.01$
RD	0.61 ± 0.02	0.63 ± 0.05	0.63 ± 0.04	$p = 0.240$; $\|r\| = 0.20$	$p = 0.527$; $\|r\| = 0.13$	$p = 0.889$; $\|r\| = 0.03$

PPA perforant pathway area, *FA* fractional anisotropy, *AD* axial diffusivity, *RD* radial diffusivity, *MP* patients with preserved memory, *MI* patients with impaired memory, *HC* healthy controls. Between-group comparisons (HC, MP, MI) were conducted using Mann-Whitney test. Cohen's |r| effect size (Cohen 1988) corresponds to small (0.10), medium (0.30) and large (0.50)

patients also showed decreased FC compared to MI patients between left hippocampus and right supramarginal gyrus ($p < 0.005$ uncorrected at voxel level; $p < 0.05$ FWE at cluster level) (Fig. 2).

Seed region: right hippocampus

MP vs. HC Compared to HC, MP patients did not show significantly decreased or increased FC between right hippocampus and other brain regions ($p < 0.005$ uncorrected at voxel level; $p < 0.05$ FWE at cluster level).

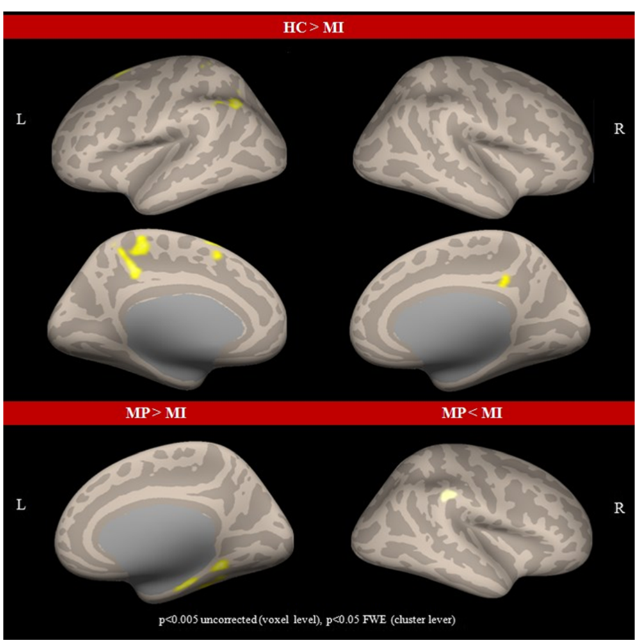

Fig. 2 Between-group comparisons for resting-state fMRI imaging connectivity maps of left hippocampus with other brain areas (HC vs. MI; MP vs. MI)

MI vs. HC There were not regions showing decreased or increased connectivity with right hippocampus in MI compared to HC.

MP vs. MI There were not regions showing decreased or increased connectivity with right hippocampus in MP compared to MI.

Discussion

Structural GM integrity

Hippocampal pathology has been previously found in MS patients with ex vivo and in vivo approaches, revealing hippocampal demyelination (Geurts et al. 2007; Papadopoulos et al. 2009), focal lesions (Roosendaal et al. 2008), gliosis (Geurts et al. 2006) and reduced glucose metabolism (Paulesu et al. 1996). Our results concerning the early detection of hippocampal structural changes in MP patients, are in line with previous neuroimaging studies (Planche et al. 2018; Sacco et al. 2015) and specifically in right hippocampus (Pardini et al. 2014; Preziosa et al. 2016; Roosendaal et al. 2010). Preziosa et al. (2016) have recently highlighted that atrophy of right hippocampus was one of the best MRI findings to discriminate MS patients with and without cognitive deficits. However, other studies did not detect hippocampal involvement in MS without memory deficits (González Torre et al. 2017). The clinical significance of early GM changes in right hippocampus is still under investigation and cannot be answered in cross-sectional designs. Nevertheless, these changes might corroborate the pattern of visual-vs-verbal memory deficits in MS. In a large sample of MS patients, visual memory (BVMT-Total Learning and Delayed Recall) was most significantly impaired compared to verbal memory and was the second most impaired cognitive processes following processing speed (i.e. SDMT) (Benedict et al. 2006). A similar pattern of more impaired visual memory compared to verbal memory was also found in a Greek sample of MS patients including different MS subtypes (Potagas et al. 2008); this finding was most evident in patients with CIS (Potagas et al. 2008) and has also been reported in probable MS patients (Achiron and Barak 2003). The presence of bilateral GM changes fits well with the current neuroimaging literature (Geurts et al. 2012) and with the neuropsychological profile of our MI patients (similar distribution of patients with verbal and visual memory deficits) who undoubtedly showed more impaired cognitive profile in general (including decreased processing speed [i.e. SDMT] and verbal fluency [i.e. WLG]).

Structural WM integrity

In addition to lesions and volume loss, MS hippocampal pathology also relates to WM microstructural abnormalities. The latter have been confirmed in a recent DTI study which focused on hippocampal DTI indices (FA, MD) in patients with CIS and definite MS (Planche et al. 2017a, b, c). Specifically, CIS patients showed significantly lower FA and increased MD compared to healthy controls; higher MD was associated with worse verbal long-term recall on SRT (Planche et al. 2017a, b, c). Of note, CIS patients with and without memory impairment based on delayed SRT recall were correctly discriminated based on hippocampal MD (Planche et al. 2017a, b, c). Previous reports also support increased diffusivity measures in bilateral hippocampi (Roosendaal et al. 2010). The previous findings provide strong support to further study intrahippocampal WM intregrity and its relation to MS memory performance, since it has been already found that limbic WM (i.e. fornix) and fronto-temporal (i.e. uncinate fasciculus) tracts are affected in MS and associated with patients' memory deficits (e.g. Kern et al. 2012; Keser et al. 2017).

To our knowledge, this is the first study that directly addresses the issue of PPA integrity in MS. The in vivo examination of the PPA has been deemed feasible due to the advantages of DTI tractography which enable the delineation of microstructural alterations along the PPA not only in healthy aging (Yassa et al. 2010) but also in several clinical groups, including patients with mild cognitive impairment and Alzheimer's disease (Kalus et al. 2006), traumatic brain injury (Christidi et al. 2011) and amyotrophic lateral sclerosis (Christidi et al. 2017; Kassubek et al. 2014). Our findings confirm the involvement of PPA in MS-related memory deficits as we found increased RD and decreased FA in left PPA in MI patients, further verifying the high sensitivity of RD in the detection of microstructural WM alterations in MS (Janve et al. 2013) and its role as a possible surrogate for demyelination (Klawiter et al. 2011; Wang et al. 2015). Based on the medium effect size for the right PPA in MP patients, our findings might also support the early detection of PPA microstructural damage in MP patients.

From a functional point of view, the PPA is the major intrahippocampal pathway which arises from layer II–III neurons of the lateral and medial entorhinal cortex, perforates the subiculum to reach the dentate gyrus and the hippocampus proper (Duvernoy et al. 2013), and contributes to encoding, consolidation, and retrieval of declarative memories (Eichenbaum 2000; Hyman et al. 1986; van Strien et al. 2009), as well as pattern separation which is the process of establishing independent and non-overlapping new memories (Yassa and Stark 2011). Of note, Planche et al. (2017a, b, c) investigated pattern separation for the first time in MS and found impaired performance in early MS patients who did not differ from healthy controls in the commonly administered BVMT. The authors interpreted their findings as an indirect evidence for dentate gyrus abnormalities which have been found in experimental MS model (Planche et al. 2017a, b, c) and also described in MS patients (Gold et al. 2010; Rocca et al. 2015) with a concomitant clinical significance (i.e. impaired memory performance).

Considering the sample size of our study and the cross-sectional design, we cannot make conclusive inferences regarding hippocampal GM changes and WM microstructural alterations in PP and the pattern of observed differences (i.e. more robust findings regarding bilateral GM changes and only left PP involvement in MI patients; right GM changes and right PPA involvement in MP patients). However, even in normal aging, the degradation of the PPA integrity has not been considered as a part of a global phenomenon that simultaneously affects other hippocampal parts (Yassa et al. 2010). Furthermore, although there are strategic lesions either in WM and GM that might contribute to patients' clinical and cognitive symptoms (Enzinger and Fazekas 2015; Charil et al. 2003; Kincses et al. 2011), the appearance of such lesions do not always follow a specific spatio-temporal pattern and there are possible differences in the mechanisms that underlie the demyelinating activity and the formation of WM and GM lesions [Prins et al. 2015]. The possibility of different pathological processes in hippocampal GM and WM has been supported by in vivo MRI findings, where increased hippocampal MD in memory-intact MS patients was not related to hippocampal volume (Roosendaal et al. 2010). Irrespectively of the underlying mechanism, mixed GM and WM hippocampal lesions are frequent and extensive in MS and were more often found in patients with cognitive decline (Geurts et al. 2007).

Functional integrity

In MS patients, FC changes between the hippocampus and several brain regions have been identified compared to HC (Barkhof et al. 2014). In fact, hippocampus has functional connections with several cortical and subcortical brain areas, including prefrontal, temporal, parietal and occipital lobes, precentral gyrus, cingulate gyrus, thalamus, putamen and cerebellum (Stein et al. 2000). Our within group analyses confirm the above-mentioned hippocampal connections (results not shown). The anatomical regions that show increased or decreased FC with the hippocampus in the between-group comparisons are also involved in MS (Comi et al. 2001) and several of them contribute to different episodic memory processes (e.g. frontal cortex; posterior cingulate; precuneus) (Desgranges et al. 1998).

To our knowledge, only three studies investigated hippocampal FC in association with MS memory deficits (González Torre et al. 2017; Hulst et al. 2015; Roosendaal et al. 2010). In MS patients with intact visuospatial memory, Roosendaal et al. (2010) observed decreased hippocampal FC that was more pronounced in patients with hippocampal atrophy. In contrary to the previous study, we didn't identify FC alterations in MP compared to HC despite the fact that we found reduced GM density in the right hippocampus. Of note, our MI patients with reduced bilateral hippocampal GM density (mostly in the left hemisphere) and disrupted left PPA integrity, showed reduced FC between left hippocampus and "hubs" of the DMN (i.e. precuneus, PCC and superior frontal gyrus). Hulst et al. (2015) identified increased FC between left hippocampus and cortical and subcortical anatomical regions in MS patients impaired mean memory performance based on the group mean z-score, which was interpreted as a maladaptive response to underlying pathological processes, possibly due to the disinhibition of DMN and other networks. Recently, González Torre et al. (2017) found increased FC between bilateral posterior hippocampi and cerebral and cerebellar areas in MS patients with impaired memory compared to MS with preserved memory and HC groups. Their findings were interpreted as a different sign of hippocampal neuropathological progression possibly related to a secondary consequence of atrophy (Baltruschat et al. 2015; Cruz-Gómez et al. 2014) and not to compensatory mechanism. Compared to MP our MI patients had increased left hippocampal connectivity with the right supramarginal gyrus, with this connection being implicated in the retrieval process of remembered vs forgotten items (Geib et al. 2017). Lower FC changes between anterior hippocampus and supramarginal gyrus have been also reported during retrieval in non-demented APOEε4 carriers compared to non-carriers (Harrison et al. 2016). Thus, hippocampal FC abnormalities seem to have determinant role in the early detection of memory deficits.

Of note, FC analysis has shown that hippocampal subregions are differentially connected with several cortical and subcortical regions, forming both positive and negative associations (Zarei et al. 2013), which might further explain patterns of increased and decreased FC changes within the same study. The anatomically distinct segments of the hippocampus along its longitudinal axis have been associated with various functional specialization (Moser and Moser 1998) and multimodal studies with cognitive measures and neuroimaging techniques have provided further evidence; for instance, better topographical memory has been associated with stronger connectivity between lingual gyrus and left anterior hippocampus (Sormaz et al. 2017).

Strengths and limitations

Strengths of our study include the multimodal design with both structural and functional neuroimaging analysis, the in vivo investigation of the PPA for the first time in MS, the well-accepted criteria for the definition of memory impairment based on cognitive tests that have been proved to be sensitive in MS and the unbiased approach with regard to FC of hippocampus (seed-to-whole brain analysis). However, there are several shortcomings, including the cross-sectional design, the small sample size, the absence of the same cognitive measures for HC, the more liberal statistical threshold for neuroimaging analysis, the merging of CIS and RRMS and the diverse previous medication.

Conclusions

In this study we highlight the early detection of structural hippocampal changes in MS even in the absence of neuropsychologically-detectable memory deficits and decreased hippocampal FC in MS patients with impaired memory performance, when both GM density and PPA integrity are affected. Hippocampal changes are of clinical relevance in the study of MS, since memory impairment is highly associated with patients' quality of life (e.g. Ruet et al. 2013) and hippocampal neuroimaging alterations may have a prognostic role in early disease stages (Planche et al. 2018). Thus, future multimodal studies with larger sample sizes, including different MS subgroups, and longitudinal design are warranted.

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Compliance with ethical standards

Conflict of interest All authors declare that they do not have any conflict of interest.

Ethical approval All procedures performed in this study which involved human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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