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BRAIN COMMUNICATIONS

Sex-specific signatures of intrinsic hippocampal networks and regional integrity underlying cognitive status in multiple sclerosis

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The hippocampus is an anatomically compartmentalized structure embedded in highly wired networks that are essential for cognitive functions. The hippocampal vulnerability has been postulated in acute and chronic neuroinflammation in multiple sclerosis, while the patterns of occurring inflammation, neurodegeneration or compensation have not yet been described. Besides focal damage to hippocampal tissue, network disruption is an important contributor to cognitive decline in multiple sclerosis patients. We postulate sex-specific trajectories in hippocampal network reorganization and regional integrity and address their relationship to markers of neuroinflammation, cognitive/memory performance and clinical severity. In a large cohort of multiple sclerosis patients ($n = 476$; 337 females, age 35 ± 10 years, disease duration 16 ± 14 months) and healthy subjects ($n = 110$, 54 females; age 34 ± 15 years), we utilized MRI at baseline and at 2-year follow-up to quantify regional hippocampal volumetry and reconstruct single-subject hippocampal networks. Through graph analytical tools we assessed the clustered topology of the hippocampal networks. Mixed-effects analyses served to model sex-based differences in hippocampal network and subfield integrity between multiple sclerosis patients and healthy subjects at both time points and longitudinally. Afterwards, hippocampal network and subfield integrity were related to clinical and radiological variables in dependency of sex attribution. We found a more clustered network architecture in both female and male patients compared to their healthy counterparts. At both time points, female patients displayed a more clustered network topology in comparison to male patients. Over time, multiple sclerosis patients developed an even more clustered network architecture, though with a greater magnitude in females. We detected reduced regional volumes in most of the addressed hippocampal subfields in both female and male patients compared to healthy subjects. Compared to male patients, females displayed lower volumes of para- and presubiculum but higher volumes of the molecular layer. Longitudinally, volumetric alterations were more pronounced in female patients, which showed a more extensive regional tissue loss. Despite a comparable cognitive/memory performance between female and male patients over the follow-up period, we identified a strong interrelation between hippocampal network properties and cognitive/memory performance only in female patients. Our findings evidence a more clustered hippocampal network topology in female patients with a more extensive subfield volume loss over time. A stronger relation between cognitive/memory performance and the network topology in female patients suggests greater entrainment of the brain's reserve. These results may serve to adapt sex-targeted neuropsychological interventions.

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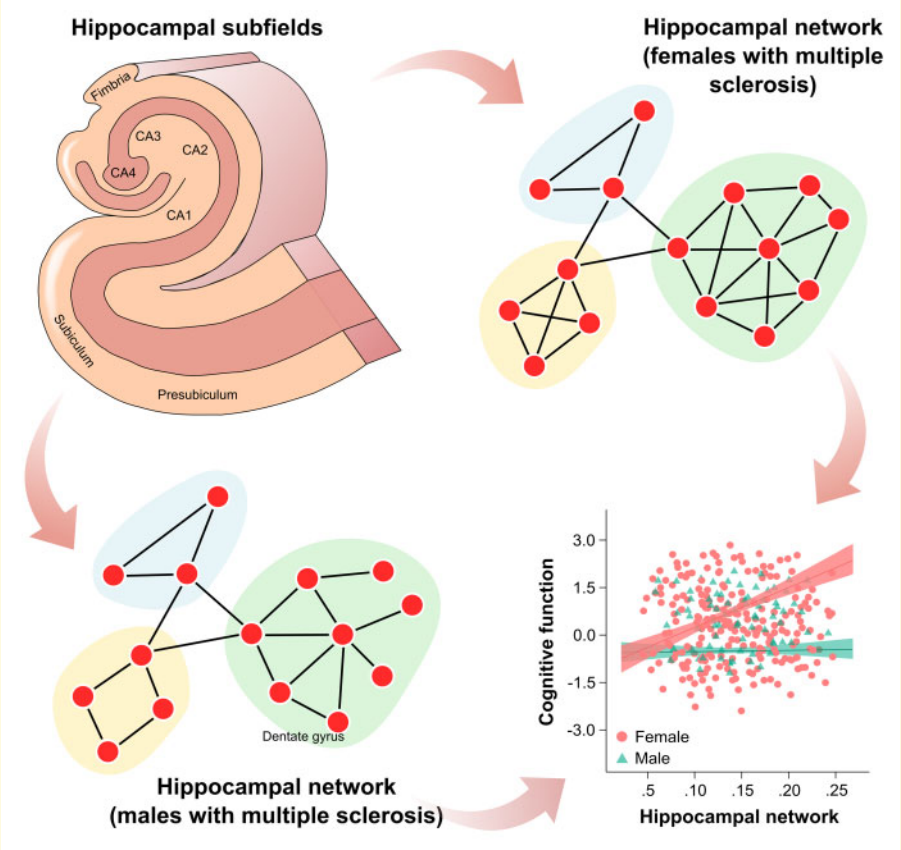
Keywords: multiple sclerosis; sex-specific signatures; hippocampal networks; hippocampal integrity; cognitive performance

Abbreviations: CA =cornu ammonis; EDSS = expanded disability status scale; FA = flip angle; FoV = field of view; FLAIR = fluid-attenuated inversion recovery; GCDG = granule cell layer of dentate gyrus; GM = gray matter; HATA = hippocampus-amygdala transition area; HS = healthy subjects; LMEM = linear mixed effects model; MP-RAGE = magnetization-prepared rapid gradient-echo; MS = multiple sclerosis; MUSIC = Multiple Sclerosis Inventory of Cognition; OASIS-3 = Open Access Series of Imaging Studies; PASAT-3 = Paced Auditory Serial Addition Test 3; RRMS = relapsing-remitting multiple sclerosis; SLIM = Southwest University Longitudinal Imaging Multimodal; TE = echo time; TI = inversion time; TR = repetition time; WM = white matter

Graphical Abstract



Introduction

Multiple sclerosis (MS) is an immune-mediated disorder of the central nervous system characterized by focal and diffuse tissue damage, presenting with heterogeneous clinical and imaging phenotypes. It has been proposed that sex might play an important role in this heterogeneity. While females are at a higher risk of MS, males are more likely to evolve to a progressive disease stage.[1] Findings from neuroimaging studies suggest lower white matter (WM) volumes in female MS patients[2] and more remarkable atrophy of subcortical grey matter (GM) structures in male patients,[3] indicating that sex might shape distinct patterns of brain tissue

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vulnerability to neuroinflammatory and neurodegenerative damage in MS. Prior evidence pointed out different trajectories of cognitive impairment in MS patients, males being more prone to cognitive decline with more cognitive domains affected than female patients.[4,5] The mechanisms of sex-specific variability in cognitive performance in MS patients are still elusive. Exploring one of the key component structures in cognitive functioning, the hippocampus might offer valuable insights into the structural correlates of cognitive performance in MS patients.

The hippocampus is a complex functionally and anatomically compartmentalized structure, with a fine-tuned intrinsic network architecture, along with widely distributed connections to other brain networks.[6,7] The integrity of the hippocampal network and subfields organization is essential for maintaining high-order cognitive functions (e.g. learning and long-term memory consolidation).[8] Vulnerability of the hippocampal formation has been recognized from the early stages of MS[9] and is related to cognitive and memory impairment.[10,11] However, measurable patterns of intrinsic hippocampal network and subfield responses to neuroinflammatory and neurodegenerative damage have not been addressed in MS. Moreover, systematic studies on sex-specific effects on hippocampal network and regional organization in MS patients are missing so far. The sex-specific hippocampal network responses can be approached through graph theoretical analysis, which is a unique tool to investigate the alterations of brain networks in MS,[12] providing more sensitive metrics to MS pathology than conventional neuroimaging measures.[13–15]

In light of the above, informing sex-specific signatures of hippocampal networks and regional structural integrity can offer valuable insights into the intrinsic hippocampal organization in MS that may underlie cognitive variability across sexes. Specifically, we test the following hypotheses: (i) morphometric network architecture of the hippocampal formation displays sex-specific differences in MS patients, (ii) regional structural integrity of the hippocampal formation follows the sex-specific network signatures and (iii) both network and regional properties distinctively relate to cognitive performance in female and male MS patients. We address these questions by constructing single-subject morphometric networks and quantifying the volumes of the hippocampal subfields based on high-resolution magnetic resonance images in a large cohort of MS patients. We apply graph theoretical analysis to model the topological organization of reconstructed hippocampal networks at the single-subject level. We relate the network and regional integrity of the hippocampus to a composite cognitive performance score in female and male MS patients. We enhance the presented framework by conducting a longitudinal study over a 2-year time period and including a control cohort of healthy subjects (HS).

Materials and methods

Study participants

Multiple sclerosis patients

From a large cohort of MS patients, prospectively enrolled at two German Neurology centres (Department of Neurology at the University Medical Center of the Johannes Gutenberg University Mainz and Department of Neurology with Institute of Translational Neurology, University Hospital of Münster), patients with available clinical and MRI data over 2 years were included ($n = 476$). The patients were diagnosed with relapsing-remitting MS (RRMS)[16] and had a disease duration of fewer than 5 years (early RRMS). The demographic and clinical characteristics of the patients are summarized in Table 1. To avoid any effects of corticosteroids on MRI-derived hippocampal volumes, patients were steroid- and relapse-free for at least 2 months before scanning.

Clinical (Expanded Disability Status Scale, EDSS), neuropsychological and MRI data were collected at baseline and at 2-year follow-up.

The ethics committee of the State Medical Board of Rhineland-Palatine, of the University of Münster and the Physicians' Chamber of Westphalia-Lippe (Ärztekammer Westfalen-Lippe, 2010-378-b-S, 2017-754-f-S) approved the study and all patients signed the informed consent prior to participation.

Neuropsychological assessment

The neuropsychological evaluation was performed by experienced neuropsychologists blinded to patients' clinical and MRI data. This included the Paced Auditory Serial Addition Test 3 (PASAT-3) and the Multiple Sclerosis Inventory of Cognition (MUSIC) test. The PASAT-3 is a cognitive test performed in MS patients to evaluate the attention, working memory and speed of information processing.[17,18] The MUSIC is a cognitive screening test aimed to assess the core cognitive domains impaired in MS—memory, attention, cognitive flexibility and information processing speed.[19] It consists of six subtests: (i) Word List Learning, (ii) Interference Word List Learning, (iii) Category Fluency Switch Condition, (iv) Modified Stroop Task and (v) Word List Recall. Memory is assessed in subtests (i) and (ii) for the immediate recall and in subtest (v) for the delayed recall.

Individual PASAT-3 and MUSIC scores were adjusted for age and education based on the normative data.[19,20] The Z-scores of PASAT-3 and MUSIC tests were averaged to calculate one composite cognitive performance score. Similarly, the Z-scores of memory-related subtests of the MUSIC, that is, Word List Learning, Interference Word List Learning and Word List Recall were averaged to calculate one composite memory performance score. The neuropsychological characteristics of the patients are illustrated in Table 1.

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Table 1 Demographic, clinical, neuropsychological and neuroimaging characteristics of the participants.

	MS ($n = 476$)			HS ($n = 110$)			MS vs. HS
	Female	Male	t/Z	Female	Male	t	
Number (%)	$n = 337$ (70%)	$n = 139$ (30%)		$n = 54$ (50%)	$n = 56$ (50%)		**$P < 0.001$*** **
Age (years)	35 ± 10	34 ± 9	$P = 0.58$*	38 ± 12	30 ± 11	**$P = 0.01$***	$P = 0.70$*
Disease duration (months) EDSS (1–10)	16 ± 11	16 ± 10	$P = 0.88$*	na	na	na	na
Baseline	1.5 (0–5)	1.5 (0–5)	$P = 0.41$**	na	na	na	na
Follow-up	1.5 (0–5)	1.0 (0–5)	$P = 0.54$**				
Composite cognitive performance score (*Z*-score)							
Baseline	−0.34 ± 1.04	−0.26 ± 0.97	$P = 0.53$*	na	na	na	na
Follow-up	−0.24 ± 1.07	−0.25 ± 1.04	$P = 0.92$*				
Composite memory performance (*Z*-score)							
Baseline	−0.08 ± 0.75	−0.01 ± 0.73	$P = 0.47$*	na	na	na	na
Follow-up	−0.09 ± 0.88	−0.002 ± 0.84	$P = 0.38$*				
T2 lesion volume (log_{10} mL)							
Baseline	0.38 ± 0.6	0.42 ± 0.6	$P = 0.58$*	na	na	na	na
Follow-up	0.46 ± 0.6	0.48 ± 0.6	$P = 0.76$*				
Hippocampal lesion volume (log_{10} mL)							
Baseline	0.55 ± 0.32	0.57 ± 0.26	$P = 0.78$*	na	na	na	na
Follow-up	0.55 ± 0.31	0.60 ± 0.25	$P = 0.40$*				

EDSS, expanded disability status scale; HS, healthy subjects; MS, multiple sclerosis.
Variables are presented as means ± standard deviation (SD) or median (range).
**P*-values derived from Student's two-tailed *t*-test (age, disease duration, composite cognitive performance *Z*-score (average of PASAT and MUSIC *Z*-scores), composite memory performance *Z*-score (average of memory-related subtests of the MUSIC test) and T2 and hippocampal lesion volumes).
***P*-values derived from Mann–Whitney U-test (EDSS).
****P*-values derived from Pearson's chi-squared test (sex). Significant *P*-values are marked in bold.

Healthy subjects

The demographic and MRI data for the HS group was searched in two open-access longitudinal MRI dataset repositories—the SLIM (Southwest University Longitudinal Imaging Multimodal) Brain Data Repository (http://fcon_1000.projects.nitrc.org/indi/retro/southwestuni_qiu_index.html Accessed 22 May 2019) and the OASIS-3 (Open Access Series of Imaging Studies) MRI database (https://www.oasis-brains.org Accessed 22 May 2019). From these two repositories, data available for a 2-year follow-up period were retrieved for 50 HS from SLIM and for 60 HS from OASIS-3 (Table 1). The SLIM database represents a long-term test–retest sample of young healthy adults in southwest China, comprising a large set of longitudinal multimodal imaging[21] from 121 subjects with three MRI sessions. The OASIS-3 represents a data compilation of more than 1000 adult participants and 2000 MRI sessions with multiple structural and functional sequences.[22]

MRI datasets

Multiple sclerosis patients

MS patients from first centre underwent MRI scanning at the Neuroimaging Center (NIC), Mainz using a 3T scanner (Magnetom Tim Trio, Siemens, Germany) with a 32-channel head coil. The imaging protocol comprised one sagittal three-dimensional (3D) T_1-weighted (T1w) magnetization prepared rapid gradient echo (MP-RAGE) and a 3D T_2-weighted (T2w) fluid attenuated inversion recovery (FLAIR) sequences with the following acquisition parameters: MP-RAGE—repetition time (TR) = 1900 ms, echo time (TE) = 2.52 ms, inversion time (TI) = 900 ms, flip angle (FA) = 9°, field of view (FoV) = 256 × 256 mm^2, matrix size = 256 × 256, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3; T2w-FLAIR—TR = 5000 ms, TE = 388 ms, TI = 1800 ms, FoV = 256 × 256 mm^2, matrix size = 256 × 256, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3.

Patients from second centre were imaged on a 3T Siemens Magnetom Prisma^{fit} scanner (Siemens, Germany) with a 20-channel head coil and the following acquisition parameters: sagittal 3D T1w MP-RAGE (TR = 2130 ms, TE = 2.2 ms, TI = 900 ms, FA = 8°, FoV = 256 × 256 mm^2, matrix size = 256 × 256, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3) and sagittal 3D T2w FLAIR (TR = 5000 ms, TE = 389 ms, TI = 1800 ms, FA = 8°, FoV = 256 × 256 mm^2, matrix size = 256 × 256, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm^3).

Healthy subjects

Participants from the SLIM database had a high-resolution T1w MP-RAGE sequence (identical with the sequence applied in the first centre) acquired on a 3T MRI scanner (Magnetom Tim Trio, Siemens, Germany). The T1w sequence parameters were: TR = 1900 ms, TE = 2.52 ms, TI = 900 ms, FA = 9°, matrix size 256 × 256, slice thickness = 1 mm, and voxel size = 1 × 1 × 1 mm^3.

From the OASIS-3 database high-resolution T1w MP-RAGE sequences acquired on a 3 T MRI scanner (Magnetom Tim Trio, Siemens, Germany) were extracted. The T1w sequence parameters: TR = 400 ms, TE =

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3.16 ms, TI = 1000 ms, FA = 8°, matrix size 256 × 256, slice thickness = 1 mm, and voxel size = 1 × 1 × 1 mm^3.

MRI processing

The study pipeline is illustrated in Fig. 1.

Longitudinal image processing

Cortical surface reconstruction and subcortical volumetric segmentation of every individual T1w image were performed using the FreeSurfer software (version 6.0, http://surfer.nmr.mgh.harvard.edu/ Accessed 01 December 2017).[23] Then, the longitudinal pipeline, which is based on the creation of an unbiased within-subject template space and image, using robust inverse consistent registration,[24] was applied. All surface models and subcortical segmentations were inspected for accuracy and manually corrected for tissue misclassification or WM errors. To avoid lesion-induced tissue misclassification errors, GM segmentation was performed after filling of T1 hypointense lesions. Cortical and subcortical GM structures were parcellated according to the Desikan–Killiany atlas.[25]

Hippocampal subfield segmentation

Subject-specific hippocampal subfields were segmented based on the preliminary T1w subcortical segmentation of the whole hippocampus by applying a Bayesian inference approach and a probabilistic atlas of the hippocampal formation.[26] This computational atlas was built upon a combination of ultra-high resolution (~0.1 mm isotropic) *ex vivo* MRI data from autopsy brains (manual delineation of the hippocampal substructures) and *in vivo* MRI data (manual annotation of the adjacent extrahippocampal structures).[26] The left and right hippocampi were each segmented into 12 subfields per brain hemisphere: parasubiculum, presubiculum, subiculum, cornu ammonis (CA) 1, CA3, CA4, granule cell layer of the dentate gyrus, hippocampus–amygdala transition area, fimbria, molecular layer, hippocampal fissure and hippocampal tail. The automated subfield segmentations were visually inspected and manually corrected where necessary. The FreeSurfer automated hippocampal subfield segmentation shows high accuracy and reliability within and across populations (healthy and diseased),[27] and high stability within and across scanner platforms.[28]

Network reconstruction and analysis

Single-subject network reconstruction

Following the hippocampal subfield segmentation into 12 subfields and prior to being entered into the network analysis, hippocampal subfield volumes for all participants (both MS and HS) were adjusted for the variations in total intracranial volume (tVol), age and scanner (Mainz, Münster, SLIM, OASIS-3) in accordance with standard protocols[29,30] using a general linear model:

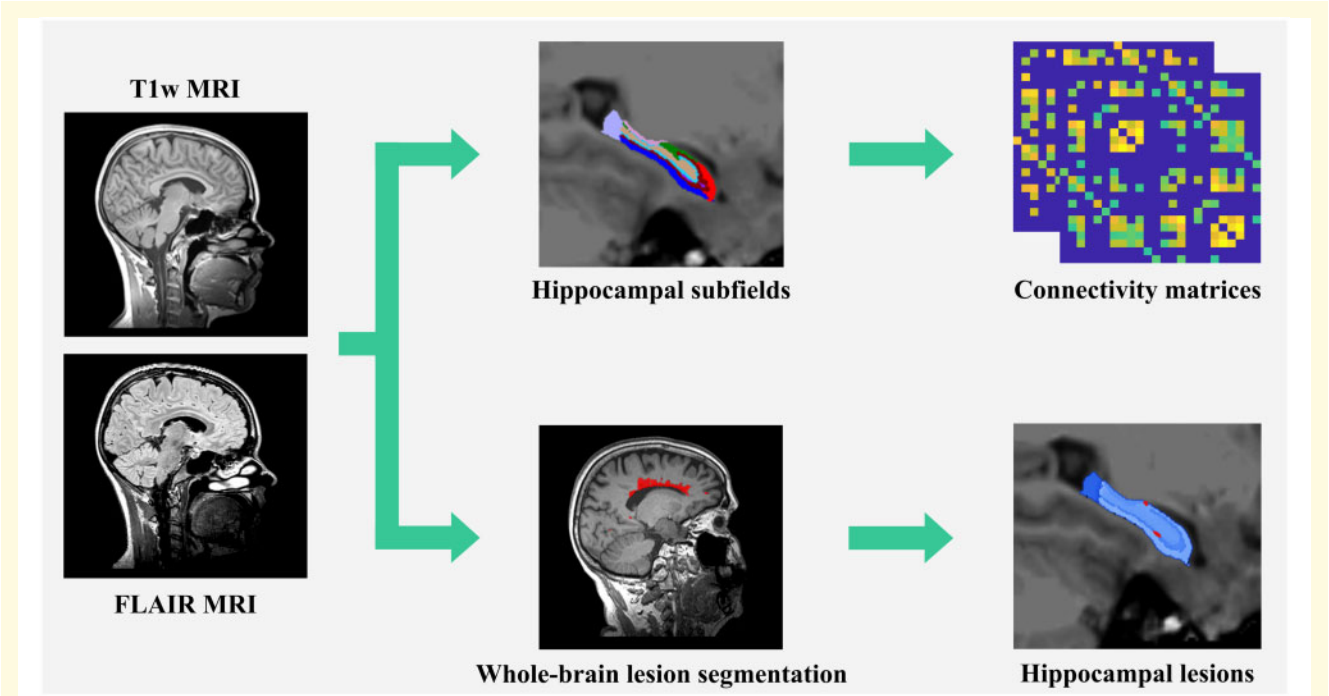

Figure 1 Study pipeline. T_1-weighted (T1w) and fluid attenuated inversion recovery (FLAIR) images were used to quantify the volumes of hippocampal subfields and to segment whole-brain lesions in multiple sclerosis patients and healthy subjects. Subsequently, from volumes of hippocampal subfields, single-subject connectivity matrices were constructed. Comparisons between females and males in hippocampal connectivity and hippocampal lesion volumes were evaluated.

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$$Vol_{adj} = \beta_0 + \beta_1\ (tVol) + \beta_2\ (Age) + \beta_3\ (Scanner) + \epsilon$$

where tVol (continuous), age (continuous) and scanner (categorical) are independent variables assumed to explain the dependent variable Vol_{adj}, β_0 is the model intercept and ϵ is the residual error. Here, the three β's are found and represent the degree of variation in Vol_{adj} associated with a variable in the model.

Following volume adjustment, morphometric hippocampal networks for each subject were constructed. Here, nodes represent individual hippocampal subfields and edges represent the volumetric similarity between each pair of subfields, computed as $Mc_{(i,\ j)} = |Vol_{adj\text{-}i} - Vol_{adj\text{-}j}|$, where Mc is the morphometric connection between the subfields of interest i and j.[31] Given 12 hippocampal subfields in each hemisphere, the procedure results in a 24×24 fully connected morphometric hippocampal network for each individual.

Identical steps were adopted to reconstruct the whole-brain GM networks (details are provided in the Supplementary Material).

Individual network topology computation

We were primarily interested in elucidating the sex-specific signatures of intrinsic hippocampal networks. The topological organization of hippocampal morphometric networks was assessed by using the Brain Connectivity Toolbox (https://sites.google.com/site/bctnet/ Accessed 10 May 2019)[32] and described in terms of clustering coefficient, modularity, local efficiency and network hub detection. The formulas applied for the calculation of network measures can be found elsewhere.[13,33,34] However, as clustering coefficient, modularity and local efficiency reflect similar features of the local network's organization and showed consistent effects in our analysis, we restricted our results to clustering coefficient.

'Clustering coefficient' is a parameter of local network organization that indicates the number of connections between the neighbouring nodes.[35] Increased clustering coefficient denotes a more strengthened local network connectivity with sparse connections to more distant nodes. 'Network hubs' represent nodes that maintain the efficient organization of the whole network and drive most of the information flows within the network.[36] Definition of a hub was based on the calculation of the betweenness centrality, defined as the number of shortest paths connecting every pair of nodes in the network and crossing through a given node. Hubs were considered those nodes, whose betweenness was two standard deviations above the mean nodal betweenness across the network regions.

Quantification of hippocampal lesions

For automated calculation of lesion volumes, we employed the lesion segmentation toolbox (LST, https://www.applied-statistics.de/lst.html Accessed 10 November 2016),[37] which is part of the statistical parametric mapping (SPM12) software (https://www.fil.ion.ucl.ac.uk/spm/ Accessed 25 November 2019). Firstly, whole-brain lesion probability maps were obtained, and afterwards, the hippocampal lesions were quantified by overlapping the hippocampal masks (derived from FreeSurfer) and the lesion probability maps. For this, the 3D FLAIR images were co-registered to the T1w images and bias-corrected. After partial volume estimation, lesion segmentation was performed with 20 different initial threshold values for the lesion growth algorithm. After visual inspection of the resulting lesion probability maps, the optimal threshold of $\kappa = 0.1$ was chosen as the optimal value for all patients. Subsequently, binary lesion maps were grown along the hyperintense voxels in FLAIR images and lesion probability maps were obtained. One advantage of the FreeSurfer subfield segmentation is that its generative nature (Bayesian inference with probabilistic atlasing) and unsupervised intensity model (i.e. not segmented based on image intensities), renders this algorithm robustness against changes in MRI contrast, including the presence of lesions.

Statistical analysis

Statistical analysis was performed using R (version 3.4.2) and RStudio (version 1.1.453), and MATLAB R2017b (Mathworks, Natick, MA, USA). Non-normally distributed data (T2 lesion volume, hippocampal lesion volume) were normalized by logarithmic (base-10) transformation. Demographic, clinical, neuroimaging and neuropsychological characteristics were compared by applying t-test, Mann–Whitney U-test, Wilcoxon or Pearson's χ^2-test, where appropriate.

To determine whether the hippocampal network topology and regional volumetry show sex-specific differences, as well as their association with clinical variables, a set of linear mixed-effects models (LMEMs) as implemented in R (lme4 package), were applied:

i. Hippocampal network parameters. The dependent term for the model was the network measure (clustering coefficient) with fixed effect terms for the group, sex, time and sex-by-time interaction and with a random intercept term for each participant. As the clustering coefficient is derived from adjusted volumes during the network analysis, the LMEM was run without covariates.
ii. Hippocampal subfield volumes. The dependent term for each model was the volume of the subfield with fixed effect terms for the group, sex, time and sex-by-time interaction and with a random intercept term for each participant. Separate models were fitted for each hippocampal subfield, which was adjusted for the ICV, age and scanner.
iii. Relationship between hippocampal network/subfield volume and clinical variables. The relationship between hippocampal network parameters and subfield volumes,

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and clinical variables (cognitive/memory performance, EDSS, disease duration) was determined by using LMEMs. Separate models were fitted for female and male MS patients with fixed effect terms for network/subfield measures and with random intercept terms for each participant, and composite cognitive performance as the dependent variable. Similar models were applied for composite memory performance, EDSS and disease duration as dependent variables. The unstandardized regression coefficients (B) and standard error of the mean (s.e.m.) are reported.

For all multivariate analyses, post-hoc tests were conducted with Bonferroni correction for multiple comparisons. A P-value of less than 0.05 was considered statistically significant.

Data availability

The data of the HS group are available at corresponding MRI repositories—SLIM (http://fcon_1000.projects.nitrc.org/indi/retro/southwestuni_qiu_index.html) and OASIS-3 (https://www.oasisbrains.org). The de-identified data of MS patients are available from the corresponding author upon a reasonable request.

Results

Multiple sclerosis patients remain clinically and cognitively preserved, while accumulating hippocampal lesions

Table 1 contains the demographic, clinical and MR imaging characteristics of MS and HS groups stratified into sex subgroups. No age differences were found between the MS and HS cohorts (35 ± 10 vs. 34 ± 15 years, $t = 0.48$, $P = 0.62$), between the MS and HS females (35 ± 10 vs. 38 ± 12, $t = -1.39$, $P = 0.16$), nor between the MS and HS males (34 ± 9 vs. 30 ± 11 $t = 1.64$, $P = 0.09$). Only, included HS males were younger than HS females ($t = 2.63$, $P = 0.01$). There were more MS females than HS females ($\chi^2 = 18.8$, $P < 0.001$), while sex distribution was similar between the two centres of the MS cohort (Mainz female/male 158/61 vs. Münster 179/78; $\chi^2 = 0.62$, $P = 0.42$), as well as between the two HS datasets (SLIM female/male 25/24 vs. OASIS-3 29/32; $\chi^2 = 0.20$, $P = 0.65$).

Within MS, there were no significant differences in baseline age, disease duration, EDSS, composite cognitive and memory performance, whole-brain T2 and hippocampal lesion volumes (all $P > 0.05$) between female and male patients (Table 1). Over the 2-year follow-up, patients' disability (as measured by EDSS), composite cognitive and memory performance showed no differences compared to baseline (all $P > 0.05$), while whole-brain T2 and hippocampal lesion volumes increased (both $P < 0.001$).

Multiple sclerosis females and males display a more clustered hippocampal network organization and lower subfield integrity

The main and interaction effects from the LMEM analysis for hippocampal network topology and subfield volumes are reported in Tables 2 and 3, respectively.

At baseline, female MS patients displayed a higher clustering coefficient compared to their healthy counterparts ($P = 0.004$) (Fig. 2). Similarly, male MS patients presented a higher clustering coefficient ($P = 0.007$) compared to HS males (Fig. 2).

Straightforwardly, at baseline, the differences in hippocampal networks were concurrent with the differences in hippocampal regional integrity. Compared to HS females, female MS patients had lower volumes across almost all hippocampal subfields—parasubiculum, presubiculum, CA1, CA3, CA4, granule cell layer of the dentate gyrus, hippocampus-amygdala transition area, fimbria, molecular layer and hippocampal tail (all $P < 0.001$), except for subiculum, hippocampal fissure and whole hippocampal volume (all $P > 0.05$) (Fig. 3).

Male MS patients showed lower volumes across many subfields—CA1, CA3, CA4, granule cell layer of the dentate gyrus, hippocampus-amygdala transition area, fimbria, molecular layer and hippocampal tail (all $P < 0.001$), except parasubiculum, presubiculum, subiculum and whole hippocampus (all $P > 0.05$) compared to HS males (Fig. 3).

Multiple sclerosis females compared to males display a more clustered hippocampal network organization and compromised regional integrity

Female MS patients in contrast to male MS patients had higher clustering coefficient ($P = 0.012$) (Fig. 2). In both female and male MS patients, the molecular layer was identified as a hub.

When comparing the subfields between female and male MS patients, females showed lower volumes of parasubiculum, presubiculum and hippocampal fissure (all $P < 0.001$) and greater volumes of the molecular layer ($P < 0.001$) (Fig. 3, Table 3). There were no significant differences in the volumes of other subfields (all $P > 0.05$). In HS counterparts, no sex-specific differences in the baseline volumes of either subfield were detectable (all $P > 0.05$) but a trend with similar direction as in MS patients for molecular layer ($P = 0.05$) and hippocampal fissure ($P = 0.08$) (Fig. 3).

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Table 2. Summary of the linear mixed effects model for hippocampal network parameters.

	MS patients				Healthy subjects				F	P
	Female		Male		Female		Male			
	B	FU	B	FU	B	FU	B	FU		
Clustering coefficient	0.132 (0.03)	0.136 (0.04)	0.131 (0.02)	0.134 (0.04)	0.117 (0.03)	0.120 (0.02)	0.117 (0.04)	0.123 (0.04)		
Group									**20.11**	**P < 0.001**
Sex									**12.10**	**P < 0.001**
Time									**31.17**	**P < 0.001**
Sex × time									**12.77**	**P < 0.001**

Variables are presented as adjusted means (standard deviation).
Significant *P*-values (Bonferroni corrected for multiple comparisons) are marked in bold.
B, baseline; FU, follow-up.

Over time multiple sclerosis females develop an even more clustered network organization along with widespread regional tissue loss

Longitudinally, female MS patients exhibited an increase in clustering coefficient ($P < 0.001$) (Fig. 2), with follow-up values higher than in male MS patients ($P = 0.014$). At follow-up, the molecular layer remained the hub node in this patient group.

These network dynamics occurred along with extensive regional volume loss across most of the subfields—presubiculum, subiculum, CA1, CA3, CA4, granule cell layer of the dentate gyrus, hippocampus-amygdala transition area, molecular layer, hippocampal tail and whole hippocampus (all $P < 0.001$) (Fig. 3). Contrary to this, over time HS females showed much less regional tissue loss compared to MS females, limited merely to parasubiculum and presubiculum (both $P < 0.001$) and sparing other subfields (Fig. 3).

Over time multiple sclerosis males develop an even more clustered network organization and less widespread regional tissue loss

Longitudinally, male MS patients presented an increase in clustering coefficient ($P < 0.001$) (Fig. 2). At follow-up, the molecular layer remained the hub node as well in male MS patients.

Hippocampal integrity was characterized by progressive volume loss in fewer hippocampal subfields compared to female MS patients—presubiculum, subiculum, CA4, granule cell layer of the dentate gyrus, fimbria, molecular layer and whole hippocampus (all $P < 0.01$) (Fig. 3). Compared to male MS patients, over time HS males manifested regional volume loss exclusively in parasubiculum and fimbria (both $P < 0.001$), rest of the regions remaining intact (Fig. 3).

Cognition is differentially related to hippocampal network architecture and structural integrity in multiple sclerosis females and males

The composite cognitive performance score was positively associated with clustering coefficient in female ($B = 2.3$, s.e.m. = 1.5, $P = 0.013$) but not in male ($B = 0.7$, s.e.m. = 2.2, $P = 0.74$) MS patients (Fig. 4). In female MS patients, the composite cognitive performance score was positively associated with the volumes of CA1 ($B = 0.001$, s.e.m. = 0.001, $P = 0.020$), CA3 ($B = 0.003$, s.e.m. = 0.003, $P = 0.010$), CA4 ($B = 0.001$, s.e.m. = 0.003, $P = 0.013$), granule cell layer of dentate gyrus ($B = 0.001$, s.e.m. = 0.002, $P = 0.022$), hippocampus-

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Table 3. **Summary of the linear mixed effects models[a] for hippocampal subfield volumes.**

	MS patients				Healthy subjects				*F*	*P*
	Female		Male		Female		Male			
	B	FU	B	FU	B	FU	B	FU		
Parasubiculum	57.8 ± 9.4	57.5 ± 9.6	65.6 ± 10.3	65.3 ± 10.8	59.8 ± 9.0	57.8 ± 8.5	66.1 ± 10.3	62.9 ± 10.4		
Group									**24.70**	***P*<0.001**
Sex									**20.30**	***P*<0.001**
Time									**39.30**	***P*<0.001**
Sex × time									2.07	*P* = 0.090
Presubiculum	293.1 ± 32.5	288.1 ± 32.7	320.5 ± 38.1	315.4 ± 36.8	303.5 ± 30.4	295.4 ± 31.7	331.7 ± 40.2	321.3 ± 37.7		
Group									**18.01**	***P*<0.001**
Sex									**9.22**	***P*<0.001**
Time									**35.85**	***P*<0.001**
Sex × time									0.88	*P* = 0.466
Subiculum	427.7 ± 42.1	424.5 ± 43.0	461.9 ± 46.3	457.2 ± 44.3	431.8 ± 41.4	427.3 ± 38.7	467.1 ± 48.8	462.4 ± 48.0		
Group									1.35	*P* = 0.216
Sex									0.45	*P* = 0.117
Time									**12.01**	***P*<0.001**
Sex × time									0.45	*P* = 0.524
CA1	619.2 ± 68.0	613.8 ± 64.1	683.7 ± 67.9	679.1 ± 67.8	638.3 ± 24.6	629.0 ± 23.6	687.1 ± 69.1	679.5 ± 69.3		
Group									**12.11**	***P*<0.001**
Sex									0.09	*P* = 0.781
Time									**15.02**	***P*<0.001**
Sex × time									**8.33**	***P*<0.001**
CA3	200.2 ± 26.9	201.0 ± 27.2	219.0 ± 27.7	220.6 ± 25.6	213.4 ± 23.7	210.8 ± 22.1	231.1 ± 25.0	228.5 ± 23.6		
Group									**28.30**	***P*<0.001**
Sex									0.33	*P* = 0.322
Time									**9.15**	***P*<0.001**
Sex × time									1.70	*P* = 0.209
CA4	248.6 ± 26.5	248.7 ± 26.1	273.3 ± 29.0	274.6 ± 27.0	261.0 ± 24.9	256.4 ± 24.4	280.3 ± 29.9	275.3 ± 28.7		
Group									**47.02**	***P*<0.001**
Sex									1.01	*P* = 0.239
Time									**23.11**	***P*<0.001**
Sex × time									1.87	*P* = 0.158
GCDG	291.2 ± 30.3	290.9 ± 30.6	322.31 ± 33.0	320.3 ± 33.4	302.3 ± 28.9	297.3 ± 28.1	323.4 ± 35.6	323.0 ± 33.9		
Group									**33.46**	***P*<0.001**
Sex									0.31	*P* = 0.602
Time									**17.90**	***P*<0.001**
Sex × time									0.86	*P* = 0.115
HATA	59.5 ± 8.4	59.3 ± 8.3	65.6 ± 8.5	64.5 ± 8.6	62.6 ± 6.7	61.5 ± 6.3	68.0 ± 7.7	67.3 ± 7.5		
Group									**51.77**	***P*<0.001**
Sex									0.88	*P* = 0.370
Time									**9.33**	***P*<0.001**
Sex × time									1.92	*P* = 0.203
Fimbria	80.3 ± 17.1	78.8 ± 16.8	86.3 ± 18.7	82.2 ± 18.8	86.4 ± 15.5	83.7 ± 16.1	99.5 ± 14.1	94.9 ± 14.3		

(continued)

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Table 3. Continued

	MS patients				Healthy subjects				*F*	*P*
	Female		Male		Female		Male			
	B	FU	B	FU	B	FU	B	FU		
Group									**19.60**	***P*<0.001**
Sex									0.77	*P*=0.590
Time									**11.51**	***P*<0.001**
Sex × time									1.22	*P*=0.104
Molecular layer	610.6 ± 57.9	606.0 ± 59.9	558.8 ± 54.1	554.6 ± 52.2	614.0 ± 61.1	612.4 ± 63.1	571.8 ± 48.7	562.4 ± 48.9		
Group									**20.92**	***P*<0.001**
Sex									**35.38**	***P*<0.001**
Time									**27.14**	***P*<0.001**
Sex × time									**12.03**	***P*<0.001**
Hippocampal fissure	147.0 ± 21.7	144.2 ± 23.1	166.7 ± 23.1	164.5 ± 21.2	142.1 ± 57.2	144.1 ± 59.1	155.6 ± 23.3	155.0 ± 20.5		
Group									**14.03**	***P*<0.001**
Sex									**9.51**	***P*<0.001**
Time									0.66	*P*=0.302
Sex × time									**5.57**	***P*=0.011**
Hippocampal tail	506.9 ± 67.2	495.1 ± 65.5	539.6 ± 69.4	533.3 ± 70.7	541.0 ± 66.4	534.5 ± 65.2	586.6 ± 58.3	577.5 ± 58.5		
Group									**29.10**	***P*<0.001**
Sex									0.11	*P*=0.663
Time									**10.08**	***P*<0.001**
Sex × time									**7.22**	***P*=0.008**
Whole hippocampus	3396.3± 309.7	3363.6± 296.8	3679.4± 336.7	3641.7± 335.7	3425.8± 284.5	3384.7± 275.7	3751.5± 355.1	3702.6± 338.9		
Group									1.01	*P*=0.199
Sex									**41.80**	***P*<0.001**
Time									**50.79**	***P*<0.001**
Sex × time									1.18	*P*=0.261

Variables are presented as adjusted means ± standard deviation.

[a]Linear models were adjusted for total intracranial volume, age and centre.

Significant *P*-values (Bonferroni corrected for multiple comparisons) are marked in bold.

B, baseline; CA1, 3, 4, cornu ammonis 1, 3, 4; FU, follow-up; GCDG, granule cell layer of dentate gyrus; HATA, hippocampus-amygdala transition area.

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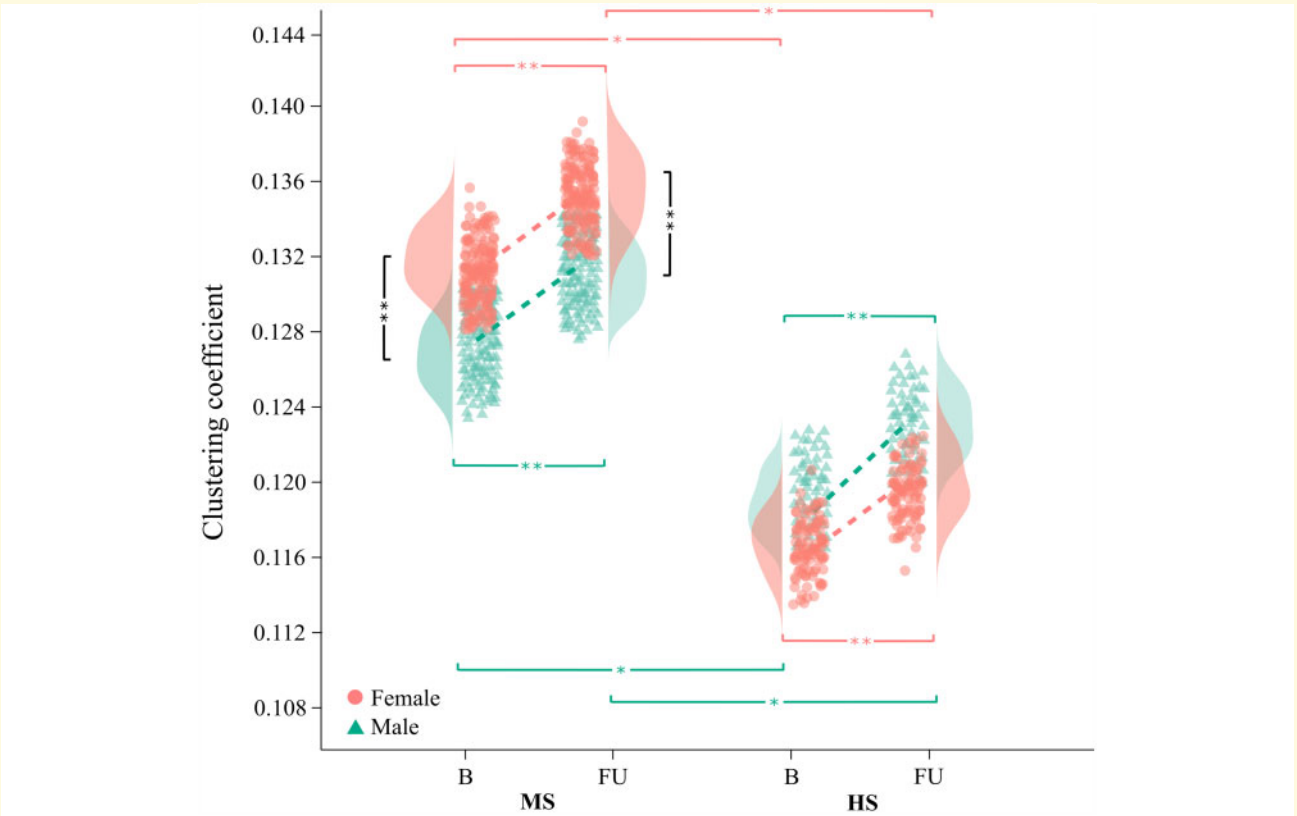

Figure 2 Sex-specific differences in hippocampal network organization. Results from the linear mixed effects model showing sex differences in network measures (clustering coefficient) in multiple sclerosis (MS) patients and healthy subjects (HS) at baseline (B) and follow-up (FU); *$P < 0.01$, **$P < 0.001$ (Bonferroni corrected for multiple comparisons).

amygdala transition area ($B = 0.008$, s.e.m. = 0.009, $P = 0.001$) and molecular layer ($B = 0.001$, s.e.m. = 0.001, $P = 0.019$) (Fig. 5). In males, the composite cognitive performance score was related to the volumes of CA1 ($B = 0.001$, s.e.m. = 0.002, $P = 0.019$) and molecular layer ($B = 0.001$, s.e.m. = 0.002, $P = 0.022$) (Fig. 5).

The composite memory performance score was positively associated with clustering coefficient in female ($B = 1.9$, s.e.m. = 1.4, $P = 0.001$) but not in male ($B = 0.8$, s.e.m. = 1.2, $P = 0.24$) MS patients (Fig. 4). In female MS patients, the composite memory performance score was associated with the volumes of presubiculum (B = -0.004, s.e.m. = 0.002, $P = 0.046$), CA3 ($B = 0.007$, s.e.m. = 0.002, $P = 0.001$), CA4 ($B = 0.005$, s.e.m. = 0.002, $P = 0.011$), granule cell layer of dentate gyrus ($B = 0.004$, s.e.m. = 0.002, $P = 0.027$) and fimbria (B = -0.007, s.e.m. = 0.003, $P = 0.012$) (Fig. 5). In males, the composite memory performance score was related only to the volume of hippocampus-amygdala transition area ($B = 0.01$, s.e.m. = 0.007, $P = 0.048$) (Fig. 5).

Additionally, a trend for a positive association between EDSS and clustering coefficient ($B = 0.003$, s.e.m. = 0.002, $P = 0.06$, Fig. 4) was observed only in female MS patients. Longer disease duration was positively associated with a higher clustering coefficient in females ($B = 0.0002$, s.e.m. = 0.0001, $P = 0.008$) but not in males ($B = 0.00004$, s.e.m. = 0.0007, $P = 0.69$, Fig. 4) MS patients. Sex-specific negative associations between EDSS, disease duration and volumes of hippocampal subfields are presented in Supplementary Material.

Hippocampal lesion volumes were not related to any of the network parameters (all $P > 0.05$) neither in female nor in male MS patients, perhaps, suggesting a higher sensitivity of hippocampal networks to neurodegeneration, rather than to inflammatory injury.

Discussion

By modelling single-subject intrinsic networks and quantifying subfield volumetric variations, we were able to detect sex-specific differences in hippocampal vulnerability in MS patients. In particular, we show that in both female and male MS patients the hippocampal network topology is more clustered compared to HS, although, more prominent in female patients. As time elapses, a similar pattern of network reorganization towards an even more clustered, and predominantly in female

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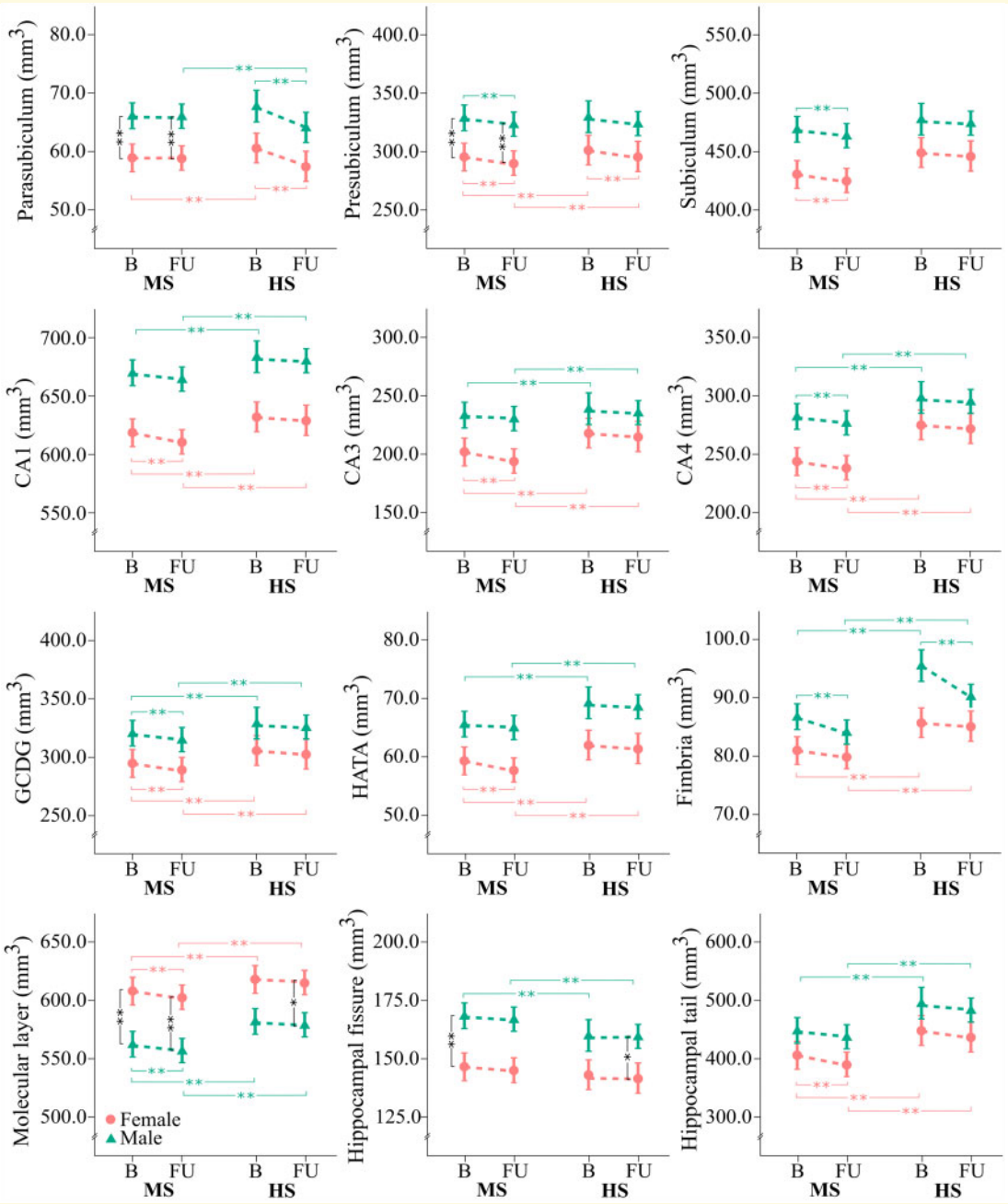

Figure 3 Sex-specific differences in hippocampal subfield volumes. Results from the linear mixed effects models showing sex differences in the volumes of hippocampal subfields in multiple sclerosis (MS) patients and healthy subjects (HS) at baseline (B) and follow-up (FU). Error bars with 95% confidence intervals are presented; *$P < 0.01$, **$P < 0.001$ (Bonferroni corrected for multiple comparisons). CA1, 3, 4 = cornu ammonis 1, 3, 4; GCDG, granule cell layer of dentate gyrus; HATA, hippocampus-amygdala transition area.

patients is retained. These network alterations occurred along with regional structural alterations. Specifically, male and female MS patients presented widespread regional subfield atrophy compared to HS, however, with a more extensive involvement observed in female patients over time. The described hippocampal network and anatomical organization were related to cognitive performance more tightly in females than in male MS patients.

Sex-specific signatures of hippocampal morphometric networks

Identifying the sex-specific phenotypes of hippocampal networks is a step forward in characterizing the network reorganization at the interplay of MS pathology and sex. The obtained measures of hippocampal network topology imply concomitantly occurring processes—increased local

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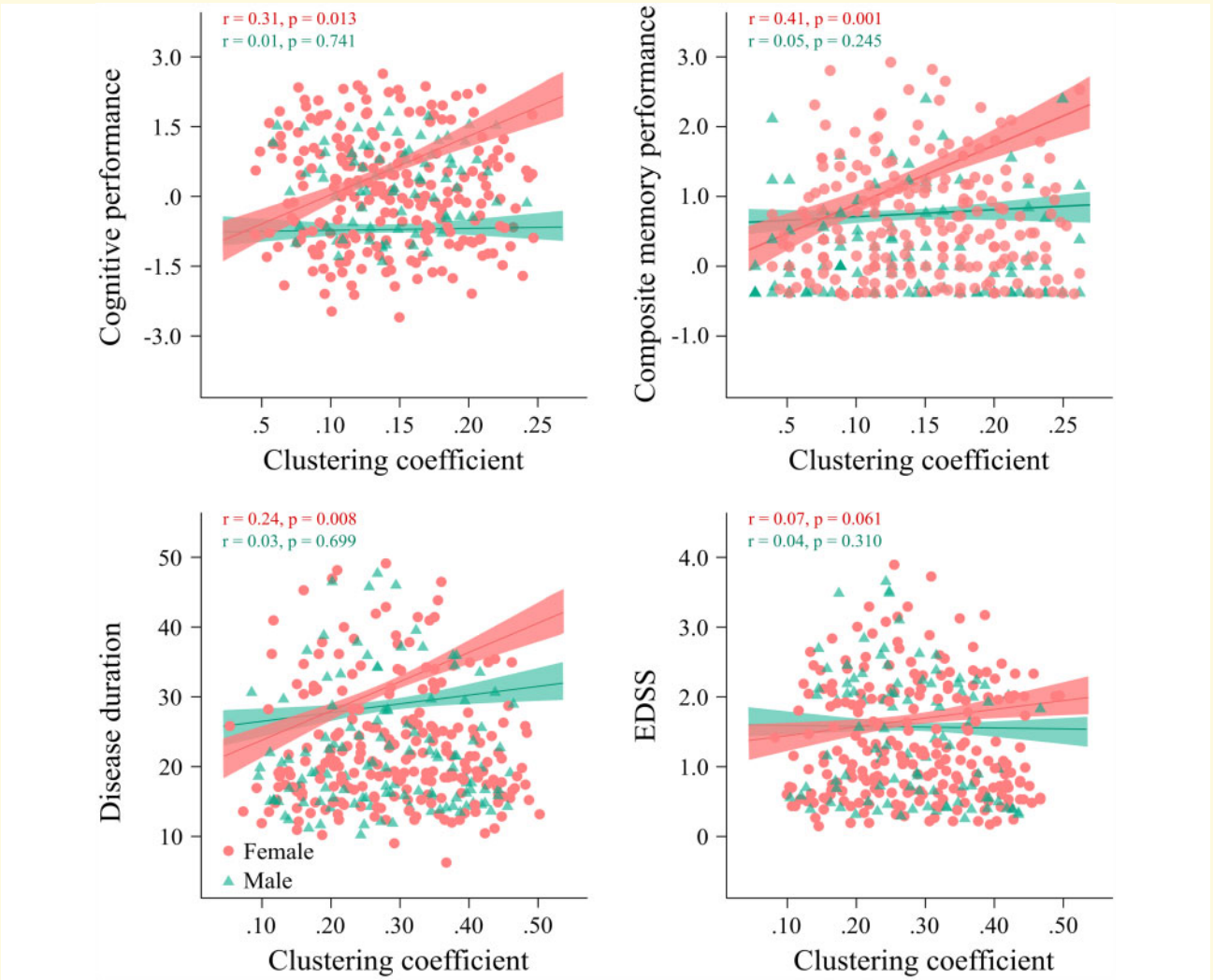

Figure 4 Sex-specific relationship between hippocampal network parameters and clinical variables. Clustering coefficient is related to composite cognitive performance, composite memory performance and disease duration in female MS patients but not in males. The slopes are significantly different between the female and male MS patients for the relation between clustering coefficient and cognitive performance ($F = 3.24$, $P = 0.04$), memory performance ($F = 4.66$, $P = 0.01$) and disease duration ($F = 3.72$, $P = 0.02$).

structural similarity and long-range structural dissimilarity, possibly related to disconnection mechanisms.[12] Hippocampal tissue remodelling elicited by MS destructive and restorative processes might underlie the observed vector in the network behaviour.[38–40] This pattern of a more clustered network organization is maintained over time regardless of sex, perhaps, as an adaptive response to ongoing localized inflammation and degeneration. This speculation is supported by the observations of more atrophied subfields with time in female patients, which have a more clustered network configuration than the male patients As it has been recently shown by our group, the increased local connectivity is possibly a compensation mechanism to structural damage aimed to reinforce the brain network functionality.[13,33,38,41] These principles of network reorganization to MS injury might be extrapolated to hippocampal networks.

Studies on sex differences in hippocampal networks in patients with MS are lacking so far. Available studies investigating whole-brain functional networks have not been able to identify any between-sex differences in MS patients.[42] Our results indicate that female MS patients display a more clustered network architecture than male patients both at baseline and after 2 years of follow-up. Several explanations for this exist. First, a more amplified compensation response of local network connectivity to a more compromised regional integrity in female patients might be hypothesized. Second, higher hippocampal connectivity in female patients might emerge from overall higher brain connectivity in females than in males as

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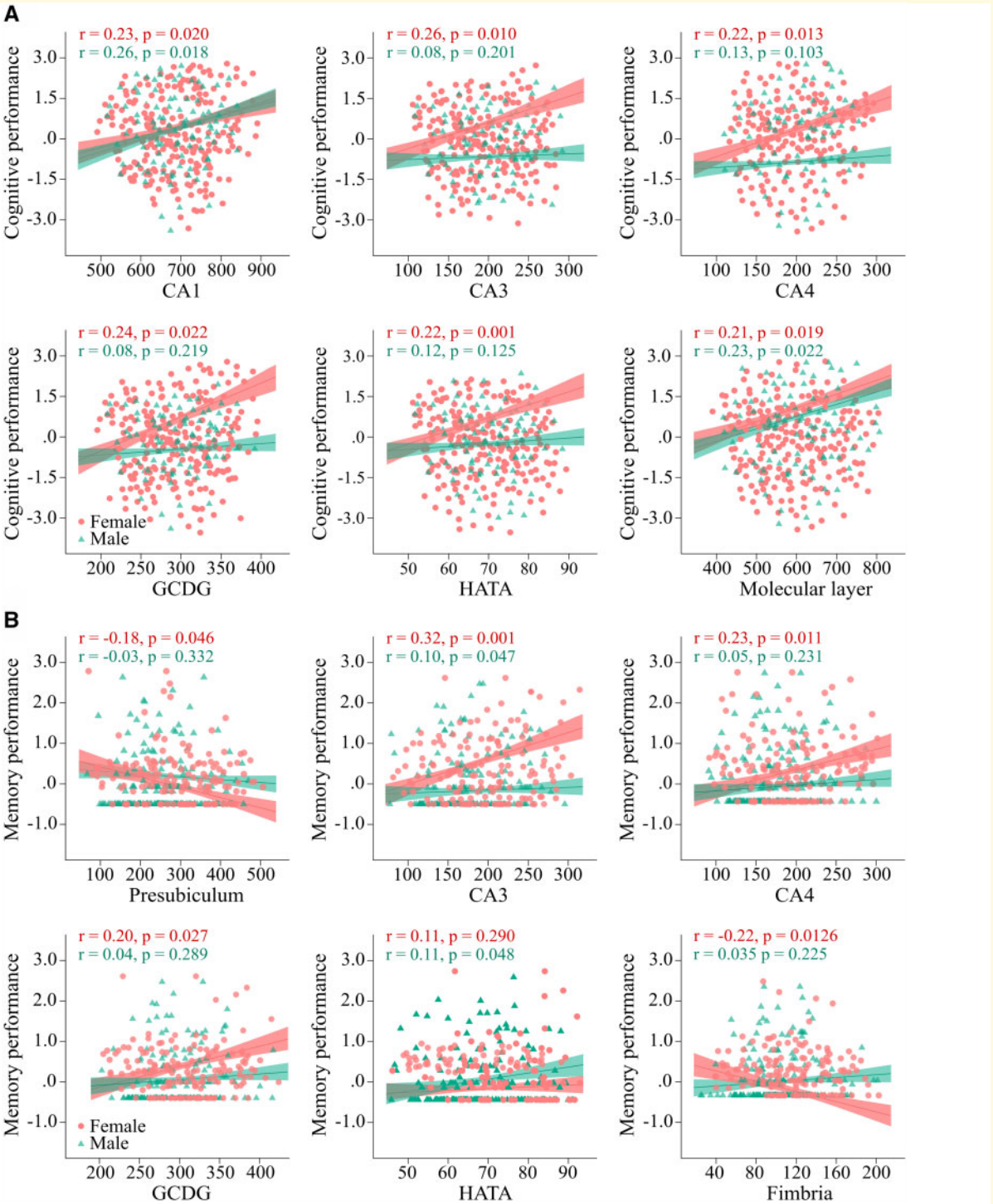

Figure 5 Sex-specific relationship between hippocampal subfield volumes and cognitive and memory performance. (**A**) Significant associations between composite cognitive performance and subfield volumes in female (cornu ammonis 1/3/4—CA1/CA3/CA4, granule cell layer of dentate gyrus—GCDG, hippocampus-amygdala transition area—HATA, molecular layer) and male (CA1, molecular layer) MS patients. The slopes are significantly different between the female and male MS patients for the relation between cognitive performance and volumes of CA3 ($F = 3.44$, $P = 0.03$), CA4 ($F = 3.20$, $P = 0.04$), CCDG ($F = 3.42$, $P = 0.03$) and HATA ($F = 3.55$, $P = 0.02$). (**B**) Significant associations between composite memory performance and subfield volumes in female (presubiculum, cornu ammonis 3/4—CA3/CA4, granule cell layer of dentate gyrus—GCDG, fimbria) and male (hippocampus-amygdala transition area—HATA) MS patients. The slopes are significantly different between the female and male MS patients for the relation between memory performance and volumes of presubiculum ($F = 3.64$, $P = 0.03$), CA3 ($F = 4.31$, $P = 0.02$), CA4 ($F = 4.99$, $P = 0.01$), CCDG ($F = 3.91$, $P = 0.02$) and fimbria ($F = 4.03$, $P = 0.02$).

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shown in this (Supplementary Material), as well as in other works.[43,44] Third, as sex steroids exert different effects on certain cortical or/and subcortical regions as parts of structural and functional networks in females and males,[45] distinct effects of sex steroids on hippocampal network architecture and functionality might be expected.[46,47] Hence, hippocampal network reorganization is an integrated, yet the sex-modulated response to physiological and pathological processes.

Sex-specific signatures of hippocampal anatomic compartments

Involvement of the hippocampal tissue integrity, occurring early during the disease course of MS, translates into reduced whole and regional hippocampal volumes.[48–50] Previous studies exploring sex effects on hippocampal volumes did not find any differences in the whole hippocampal volume between female and male MS patients.[3,51] We show that both female and male MS patients have lower volumes in almost all hippocampal subfields compared to healthy females and males. Several pathophysiological processes, ranging from inflammatory demyelination, decreased dendritic and axonal density to neuronal cell loss and gliosis have been proposed as primers of regional hippocampal atrophy in MS.[52,53] One must be confident that additionally to sex, variation in the volumes of distinct hippocampal subfields might be attributed to different factors that selectively impact the integrity of the subfields, i.e. age,[54,55] brain volume changes during the life span,[56] cardiovascular, pro-inflammatory and APOEε4 risk factors.[57]

Sex-specific differences in regional hippocampal microstructural and physiological properties in response to acute and chronic MS neuroinflammatory damage have not been previously characterized in MS patients. In this respect, several observations might support our findings: (i) animal models of MS show sex differences in immune cell and cytokine repertoire and disease severity,[46] (ii) regional hippocampal integrity relates to cerebrospinal fluid (CSF) inflammatory markers impacting synaptic plasticity and cognitive function in MS patients,[58] and (iii) high GM atrophy rates in MS patients are associated with high CSF levels of immunoglobulins.[59] By comparing female and male MS patients, we show that regional volumetric differences are evident in particular subfields, with lower volumes of pre- and parasubiculum but higher volumes of the molecular layer in female patients. However, over time the neurodegenerative process entrains the majority of hippocampal subfields with more regions affected in female patients. The reasons for these results remain unclear but a sex-specific imbalance between neural damage and repair processes is driven by a multifactorial interaction between immune cells, inflammatory mediators and sex steroids on one side and neuronal cell, axonal and myelin turnover on the other side, is very likely.[46]

Hippocampal network and anatomical correlates of cognitive performance

Existing evidence strongly suggests that females and males differentially recruit hippocampal networks during cognitive tasks.[60] Previous studies claimed that male MS patients perform worse than female patients in several cognitive domains, including processing speed, verbal memory and executive functioning.[3,61] However, in our cohort, cognitive and memory performance scores did not differ between female and male patients, perhaps, due to the early disease stage or the sensitivity of applied screening tests.[62] Positive associations between higher clustering and better cognitive and memory performance scores only in female MS patients suggests that females might integrate more efficiently the hippocampal networks into the global brain networks[63] mediating the information processing and superior verbal memory in females compared to males,[64,65] respectively. Alternatively, females might activate more limbic networks, including the hippocampal and prefrontal networks, whereas males recruit more distributed networks during cognitive tasks relying on working memory performance.[66]

The here depicted associations between hippocampal subfield integrity and cognitive and memory performance scores support previous findings showing that along with cortical and subcortical GM structures[67] the hippocampus is also involved in information processing speed, cognitive flexibility and reserve.[64,68,69] We extend these data and show that cognitive and memory performance scores are related to the volumes of more subfields in female MS patients than in male patients. These findings endorse the obtained correlations between the network topology and cognitive and memory performance scores in female MS patients, suggesting that females rely more on hippocampal networks than males during the execution of cognitive tasks involving processing speed and verbal memory. Most of the previous studies in MS reported mainly the relation between cognitive and memory impairment and the integrity of the CA1 region.[48–50] Thus, our work represents a step forward into a more detailed characterization of the correlates of cognitive and memory performance in MS.

Strengths, limitations and perspectives

The following strengths of the current study are worth to be mentioned. First, the inclusion of a large cohort of MS patients with closely matched healthy controls. Second, the

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longitudinal design of the study allowed us to investigate the time effects on the variables. Third, by modelling the subject-specific morphometric networks, individual trends of network dynamics have been captured. Several limitations apply to this study. The relatively short follow-up period of two years, which was, however, enough to capture the sex-specific trajectories of hippocampal network reorganization and regional atrophy. The employed here neuropsychological tests are screening tools and might be less sensitive in detecting sex-specific variations in cognitive impairment at early disease stages of MS.[62] One can assume that the obtained results could be biased by including more female than male MS patients. Nevertheless, this bias was mitigated by applying mixed-effects models and comparing MS patients to their healthy sex counterparts. Considering the potential effects of the type of scanner and acquisition parameters on the accuracy of hippocampal segmentation, we included the type of scanner as a confounding factor. In addition, hippocampal segmentation performed in FreeSurfer shows high reliability across different scanner platforms.[28] Given the interethnic differences in brain morphometry,[70,71] inclusion in our study of HS of different ethnicities might have influenced our observations. This and other aspects of sex-specific differences in the hippocampal organization might be addressed in target works. As the spatial location of lesions predetermines the patterns of GM pathology,[72] identification of sex-specific responses of hippocampal networks and regional integrity to the spatial distribution of intrahippocampal lesions might be of interest.

Conclusions

Our observations suggest a more clustered pattern of hippocampal network organization in females than in male MS patients that are preserved with the disease evolution. Sex-specific network reorganization follows the structural pattern of more extensive atrophy of hippocampal compartments in female MS patients over time. The differential relation of cognitive performance to hippocampal network and regional substrates might explain the variability in cognitive functioning and advance the development of personalized sex-targeted cognitive rehabilitation strategies, aimed to combat the accrual of cognitive burden.

Supplementary material

Supplementary material is available at *Brain Communications* online.

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Competing interests

The authors report no competing interests.

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