

Correlations between hippocampal functional connectivity, structural changes, and clinical data in patients with relapsing-remitting multiple sclerosis: a case-control study using multimodal magnetic resonance imaging

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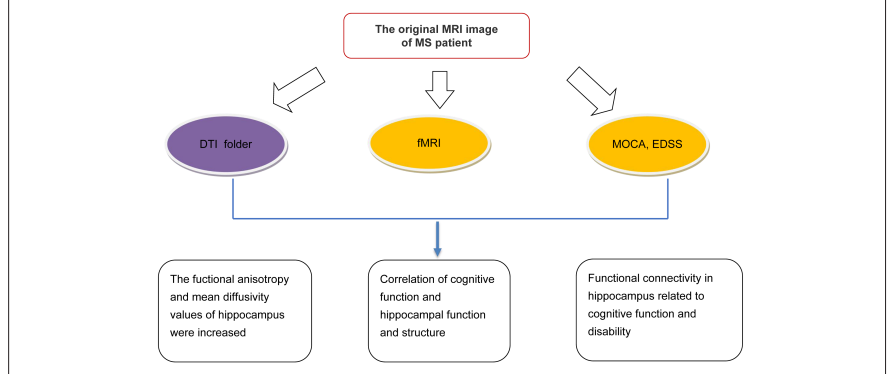
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Graphical Abstract A combination of hippocampal function and structure change correlation to cognitive function and disability in relapsing-remitting multiple sclerosis (MS)



Abstract

Multiple sclerosis is associated with structural and functional brain alterations leading to cognitive impairments across multiple domains including attention, memory, and the speed of information processing. The hippocampus, which is a brain important structure involved in memory, undergoes microstructural changes in the early stage of multiple sclerosis. In this study, we analyzed hippocampal function and structure in patients with relapsing-remitting multiple sclerosis and explored correlations between the functional connectivity of the hippocampus to the whole brain, changes in local brain function and microstructure, and cognitive function at rest. We retrospectively analyzed data from 20 relapsing-remitting multiple sclerosis patients admitted to the Department of Neurology at the China-Japan Union Hospital of Jilin University, China, from April 2015 to November 2019. Sixteen healthy volunteers were recruited as the healthy control group. All participants were evaluated using a scale of extended disability status and the Montreal cognitive assessment within 1 week before and after head diffusion tensor imaging and functional magnetic resonance imaging. Compared with the healthy control group, the patients with relapsing-remitting multiple sclerosis had lower Montreal cognitive assessment scores and regions of simultaneously enhanced and attenuated whole-brain functional connectivity and local functional connectivity in the bilateral hippocampus. Hippocampal diffusion tensor imaging data showed that, compared with the healthy control group, patients with relapsing-remitting multiple sclerosis had lower hippocampal fractional anisotropy values and higher mean diffusivity values, suggesting abnormal hippocampal structure. The left hippocampus whole-brain functional connectivity was negatively correlated with the Montreal cognitive assessment score ($r = -0.698$, $P = 0.025$), and whole-brain functional connectivity of the right hippocampus was negatively correlated with extended disability status scale score ($r = -0.649$, $P = 0.042$). The mean diffusivity value of the left hippocampus was negatively correlated with the Montreal cognitive assessment score ($r = -0.729$, $P = 0.017$) and positively correlated with the extended disability status scale score ($r = 0.653$, $P = 0.041$). The right hippocampal mean diffusivity value was positively correlated with the extended disability status scale score ($r = 0.684$, $P = 0.029$). These data suggest that the functional connectivity and presence of structural abnormalities in the hippocampus in patients with relapse-remission multiple sclerosis are correlated with the degree of cognitive function and extent of disability. This study was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University, China (approval No. 201702202) on February 22, 2017.

Key Words: cognitive impairment; diffusion tensor imaging; fractional anisotropy; functional connectivity; functional magnetic resonance imaging; hippocampus; local consistency; low frequency oscillation amplitude; mean diffusivity; multiple sclerosis; neurodegeneration

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Introduction

Cognitive deficits adversely affect the daily function of multiple sclerosis (MS) patients, especially in terms of quality of life of indices such as independence, social inclusion, and emotional stability (Chan et al., 2017; Oset et al., 2020; Gaetani et al., 2021). Changes in cognitive function may be related to alterations in brain structure and function (Ontaneda and Fox, 2017; Karavasilis et al., 2019; Lashkari et al., 2021). Functional magnetic resonance imaging (fMRI) can be used to generate dynamic physiological information via blood oxygen level-dependent (BOLD) signals (Filippi and Rocca, 2013). In regard to cognition, MS patients initially exhibit increased connectivity as a manifestation of compensation mechanisms. However, brain connectivity declines as the disease progresses (Penner et al., 2003). fMRI studies have reported on the reorganization of neural functional activity during cognitive tasks in MS patients (Mainero et al., 2004; Margulies et al., 2010). In studies of spontaneous brain activity, voxel-based regional homogeneity (ReHo) analysis and amplitude of low-frequency fluctuation (ALFF) analysis reflect the temporal synchrony of neuronal activities (Chen et al., 2017) and the measurement of local resting-state fMRI time course cortical low-frequency oscillation amplitude values (Duff et al., 2008; Zuo et al., 2010), respectively. Diffusion tensor imaging (DTI), where water molecule motion is characterized by applying multiple diffusion gradients (Sbardella et al., 2013; Bagnato et al., 2019; Stock et al., 2020), is ideal for identifying pathological changes in cognition in MS patients (Vrenken et al., 2006; Yu et al., 2012). Microscopic features have been used to distinguish MS lesions with more axonal damage from lesions that are hyperintense in T2-weighted sequences (Bagnato et al., 2019).

The increasing evidence for a pure amnesic-like profile in MS supports the role of hippocampal formation in MS episodic memory function (Karavasilis et al., 2019). The hippocampus, as an important brain structure for memory, undergoes microstructural changes in the early stage of MS. Microstructural damage measured by DTI has been observed in patients with clinically isolated syndrome and RRMS, and the MRI findings were strongly correlated with long-term recall (Planche et al., 2017). Moreover, in patients examined two to 6 months after clinically isolated syndrome, performance on learning trials was correlated with hippocampal volume (Planche et al., 2018). Here, we hypothesized that there would be a correlation between changes in hippocampal structure and function in patients with RRMS. We used DTI voxel-based analysis and multimodal fMRI to investigate the relationship between structural and functional changes in the hippocampus and cognitive deficits and other symptoms in patients with RRMS.

Participants and Methods

Participants

This was a retrospective case control study. We retrospectively collected data from 20 patients with RRMS who were admitted to the Department of Neurology at the China-Japan Union Hospital of Jilin University, China from April 2015 to November 2019. The patients included 5 men and 15 women with an average age of 43.00 ± 12.25 (27–64) years old. The mean disease duration was 6.50 ± 2.27 (4–12) years, and the average education level was 10.70 ± 3.09 (5–15) years. A total of 16 physically healthy volunteers from the China-Japan Union Hospital were retrospectively recruited for the healthy control group (HC), including 8 men and 8 women with an average age of 41.37 ± 11.27 (27–56) years old. The average educational level of the HC group was 10.88 ± 3.52 (5–16) years.

All subjects were evaluated using the extended disability

status scale (EDSS) (Wingerchuk et al., 2006) and the Montreal cognitive assessment (MoCA) (Nasreddine et al., 2005) within 1 week before and after the fMRI. The clinical data, including patient disease duration, EDSS scores, and MoCA scores were recorded by two experienced neurologists (YL and JBG). The EDSS quantifies disability by assigning a functional system score for the pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, and other functional systems. The following cognitive assessments were performed for all subjects using the MoCA: visuospatial/exclusion, naming, memory, attention, language, abstraction, and delayed recall. This study was approved by the Ethics Committee of the China-Japan Union Hospital (approval No. 2017022202) on February 22, 2017, and all participants signed informed consent forms. This study was performed in strict accordance with the *Declaration of Helsinki* formulated by the World Medical Association.

Inclusion criteria for RRMS patients

The patient inclusion criteria were: (1) McDonald's 2010 revised diagnostic criteria for MS (Polman et al., 2011) and the 2016 European MS Magnetic Resonance Imaging Multicenter Collaborative Group (MAGNIMS) MS MRI diagnostic criteria (Filippi et al., 2016). (2) 22–65 years of age (to avoid the influence of physiological atrophy); (3) no serious mental illness or neurological disease history other than MS; (4) Chinese as a primary language; (5) right-handedness; (6) a MRI head scan conducted using the same scanner at our institution; (7) relapse-free status without steroid treatment for at least 6 weeks and no current disease modifying treatments.

Exclusion criteria for RRMS patients

The patient exclusion criteria were: (1) clear intracranial infarction, intracranial space-occupying lesion, or other lesions that could affect the outcome of the study; (2) severe cardiopulmonary diseases; (3) age over 65 years.

Inclusion criteria for healthy volunteer controls

The inclusion criteria for healthy volunteers were: (1) right-handedness, matched with the patients in terms of age, sex, and education level; (2) good physical health status, with no neurological diseases, underlying systematic diseases affecting the nervous system, neurologically positive symptoms, or abnormalities observed on a routine MRI scan.

Exclusion criteria for healthy volunteer controls

The exclusion criteria for the healthy volunteers were: (1) clear intracranial infarction, intracranial space-occupying lesion, or other lesions that could affect the outcome of the study; (2) severe cardiopulmonary disease; (3) age over 65 years.

The RRMS patients were relapse-free and without steroid treatment for at least 6 weeks prior to scanning and were not undergoing any disease modifying treatment. Volunteers in the HC group were evaluated using the EDSS (Wingerchuk et al., 2006) and the MoCA (Nasreddine et al., 2005) within 1 week before and after the fMRI.

Image acquisition

All MRI scans were performed on the same Siemens Trio Tim 3.0T MR scanner (Siemens Medical Systems, Erlangen, Germany) from April 2015 to November 2019. The standard protocol consisted of three sequences: blood oxygen level-dependent functional magnetic resonance imaging (BOLD-fMRI) (echo time = 30 ms, repetition time = 2500 ms, flip angle = 90°, slice thickness = 3.0, slice number = 43, field of view = 210 mm), DTI (echo time = 95.0 ms, repetition time = 3700 ms, slice thickness = 4.0, slice number = 25, and field of view = 220 mm), and 3DT1 weighted imaging (echo time/

repetition time = 2.98/5000 ms, slice thickness/gap = 1.0/0 mm, field of view = 256 mm). The participants were instructed to close their eyes while staying awake and to try not to think about anything.

Data processing and analysis

We used the REST_V1.8 resting-state fMRI data analysis toolkit (<http://www.restfmri.net>) in MATLAB7.14 (R2012a) (Math Works, Inc. Natick, MA, USA, <http://www.mathworks.com/products/matlab>) to divide the bilateral hippocampus into regions. The hippocampal regions were identified using an automated anatomical labeling template (Tzourio-Mazoyer et al., 2002). The brain regions and hippocampal data were confirmed in Montreal Neurological Institute (MNI) space using MRICron (<http://www.sph.sc.edu/comd/rorden/mricron/>) (automated anatomical labeling 37 and 38). MNI space is a coordinate system based on a series of MRI images of healthy participants (Fjalldal et al., 2018). The left and right hippocampi were sampled at $3 \times 3 \times 3 \text{ mm}^3$. The voxels of the re-sampled left and right hippocampi were 273 and 288, respectively. The collected image data were classified and analyzed using RadiAnt DICOM Viewer (<http://www.radiantviewer.com>) software, and the BOLD-fMRI, 3DT1 weighted imaging, and DTI sequence data were extracted from the image data of each subject. We used a data processing assistant for resting-state fMRI (DPARSM, <http://www.restfmri.net>) in MATLAB to pre-process the BOLD-fMRI data, and REST software for functional connectivity (FC) analysis, ReHo analysis, and analysis. We used Functional MRI Software Library software based on the Pipeline for Analyzing Brain Diffusion Images (PANDA, <http://www.nitrc.org/projects/panda/>) to process the DTI data and obtain bilateral hippocampal FA and MD Values. The voxel-based analysis method in Statistical Parametric Mapping (SPM8, <http://www.fil.ion.ucl.ac.uk/spm/software/Spm8/>) was used to analyze the data.

Quality control

The patient clinical assessment data, including disease duration, EDSS score, and MoCA score, were recorded by experienced neurologists (YL and JBG).

Outcomes

All subjects underwent the EDSS and MoCA within 1 week before and after the MRI.

MoCA: Cognitive functions were examined using the MoCA (Nasreddine et al., 2005). The following cognitive assessments were performed for all subjects: (1) visuospatial, (2) naming, (3) memory, (4) attention, (5) language, (6) abstraction, (7) delayed recall. A score of 26 or greater is considered normal.

EDSS: Disability status was examined using the EDSS. The EDSS quantifies disability by assigning a functional system score to each of eight functional systems: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, other.

Statistical analysis

The demographic variables and clinical scores of the RRMS patients and HC subjects were statistically analyzed using SPSS 20.0 software (IBM, Armonk, NY, USA). Two-sample *t*-tests were used to evaluate differences in age, duration of disease, years of education, and clinical assessment scores between the MS patients and HC volunteers. The chi-square test was used to compare the sex distribution of the two groups. The data are expressed as the mean \pm standard deviation. For non-normally distributed data (EDSS score), the median (interquartile range) is provided. Independent two-sample *t*-tests were performed for the standardized FC, ReHo, and ALFF maps using REST software with age, sex, and years of education as co-variables. Multiple corrections were carried

out using AlphaSim (<https://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf>), for voxels > 5 . *P*-values less than 0.05 indicated statistical significance. The DTI parameters were analyzed using SPM8 software (<https://www.fil.ion.ucl.ac.uk/spm/>) for the two-sample *t*-tests between groups. FDR was used for multiple correction, for voxels > 10 .

We initially evaluated the distribution of the values for each parameter. After testing, all of the values had a normal distribution, so the Pearson correlation coefficient was adopted. The resting-state fMRI measurements (FC, ReHo, and ALFF values) and DTI measurements (FA and MD values) in the RRMS group were evaluated to assess correlations with the clinical scores.

Results

Participant demographics and clinical data

We assessed data from a total of 20 RRMS patients. All patients underwent clinical assessments and structural MRI. After statistical analysis, the sex, age, and education level were matched between the two groups. The median EDSS score (range) was 2.50 (1.0–4.50). Compared with the HC group, the MoCA scores in the RRMS group were significantly lower ($P < 0.001$), while there were no between-group differences in the other variables ($P > 0.05$; **Figure 1** and **Table 1**).

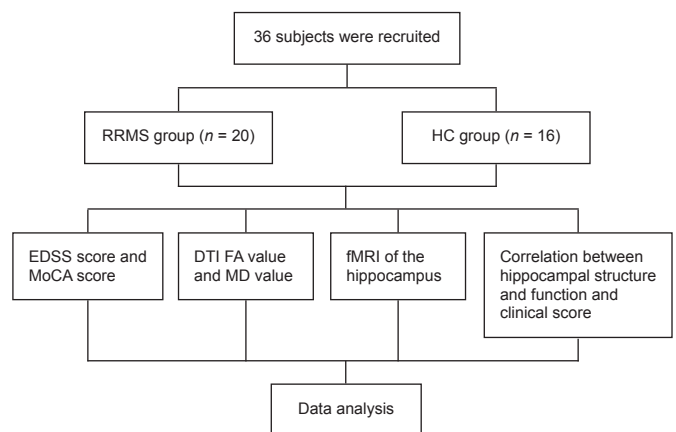


Figure 1 | Flow chart of study procedure.

DTI: Diffusion tensor imaging; EDSS: expanded disability status scale; FA: fractional anisotropy; fMRI: functional magnetic resonance imaging; HC: healthy control; MD: values and higher mean diffusivity; MoCA: Montreal cognitive assessment; RRMS: relapsing-remitting multiple sclerosis.

Table 1 | Demographics and clinical data from the RRMS and healthy control groups

Variables	RRMS group (n = 20)	Healthy control group (n = 16)	<i>P</i> -value	<i>t</i> / χ^2 value
Age (yr)	43.00 \pm 12.25	41.37 \pm 11.27	0.08	1.80
Sex (male/female, n)	5/15	8/8	0.83 [#]	0.79
Disease history (yr)	3.50 \pm 1.27	/	/	/
Education level (yr)	10.70 \pm 3.09	10.88 \pm 3.52	0.71	-1.89
Median EDSS (score)	2.50(1.00–4.50)	/	/	/
MoCA (score)	25.20 \pm 0.92	27.75 \pm 1.28	0.001 [*]	-4.28

Data are expressed as the mean \pm standard deviation. For non-normally distributed data, the median (interquartile range) is provided. *Two-sample *t*-tests; #Chi-squared test. EDSS: Expanded disability status scale; MoCA: Montreal cognitive assessment; RRMS: relapsing-remitting multiple sclerosis.

Differences in FC between the groups

Compared with the HC group, the whole-brain FC values of the bilateral hippocampi in the RRMS group indicated that there were both enhanced and attenuated regions. The left hippocampal regions with attenuated FC on the left and right sides of the brain were the cingulate gyrus, limbic lobe, and

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right brain stem; while the regions with enhanced whole-brain FC were the right cerebellum (posterior cerebellum), bilateral temporal lobe, suboccipital lobules, frontal lobe (prefrontal lobe, frontal gyrus), amygdala, lateral globus pallidus, and lentiform nucleus (**Table 2** and **Figure 2**). The right hippocampal regions with attenuated FC on the left and

right sides of the brain were the right brain stem, limbic lobe, middle temporal gyrus, para-hippocampal gyrus, and left frontal lobe; while the regions with enhanced whole-brain FC were the right cerebellum (posterior cerebellum), left occipital lobe, left frontal lobe, insula, and parietal lobe ($P < 0.001$, corrected by AlphaSim, voxel > 5 ; **Table 3** and **Figure 2**).

Table 2 | Brain regions in which the FC with the left and right side of the brain in the left hippocampus differed between the RRMS and healthy control groups

Brain region (automated anatomical labeling template)	Voxel (mm ³)	MNI coordinates			T value
		X	Y	Z	
Left side					
Temporal lobe (middle temporal gyrus, superior temporal gyrus)	5	-42	6	-30	-5.166
Temporal lobe (inferior temporal gyrus), fusiform gyrus	5	54	-6	-30	-4.451
left cerebellum (posterior cerebellar lobe), fusiform gyrus, anterior cerebellar lobe, medial occipital gyrus	48	-33	-78	-21	6.178
Amygdala, lateral globus pallidus, lentiform nucleus	9	-21	-6	-9	7.043
Frontal lobe, precentral gyrus	161	-48	39	3	7.672
Temporal lobe (middle temporal gyrus)	44	-57	-51	-3	5.526
Insula	7	-42	-3	0	6.489
Frontal lobe, insula	15	-30	18	-3	4.953
Occipital lobe	5	-39	-84	6	4.300
Frontal lobe, precentral gyrus	6	-51	-6	6	5.158
Occipital lobe, occipital lobe	9	-24	-90	9	5.312
Occipital lobe	21	33	-84	12	5.686
Parietal lobe, postcentral gyrus, temporal lobe (transverse temporal gyrus), frontal lobe	8	-60	-12	15	5.311
Frontal lobe, caudate nucleus	5	-24	-24	27	-5.812
Precentral gyrus, postcentral gyrus, frontal lobe, parietal lobe	21	57	-3	24	5.366
Occipital lobe, occipital lobe	7	-9	-90	18	4.607
Superior parietal lobe, precuneus	63	-18	-69	60	7.266
Frontal lobe, precentral gyrus, parietal lobe, postcentral gyrus, paracentral lobule	43	-24	-27	60	6.429
Hypothalamus, midbrain, left brain stem, limbic lobe, hippocampus, mammillary body	13	-21	-27	-6	6.678
Right side					
Posterior cerebellar lobe, cerebellar tonsil, right brain stem	34	6	-42	-45	-9.504
Limbic lobe, para-hippocampal gyrus	5	-18	3	-36	4.998
Temporal lobe (inferior temporal gyrus), fusiform gyrus	5	54	-6	-30	-4.451
Temporal lobe (middle temporal gyrus, inferior temporal gyrus), fusiform gyrus, right cerebellum (anterior cerebellar lobe)	42	45	-54	-21	7.193
Occipital (medial occipital gyrus), temporal lobe, posterior cerebellar lobe	17	45	-66	-15	5.224
Parietal lobe, postcentral gyrus, frontal lobe, precentral gyrus	6	60	-12	15	4.342
Frontal lobe (inferior frontal gyrus)	34	54	21	27	5.330
Limbic lobe, posterior cingulate gyrus	7	6	-57	24	-5.215
Frontal lobe	47	-39	12	30	8.507
Occipital lobe, temporal lobe, angular gyrus	7	33	-78	27	5.274
Limbic gyrus, cingulate gyrus, medial frontal lobe	33	0	18	45	6.895

RRMS patients (corrected $P < 0.001$ using AlphaSim voxel > 5). T value < 0 , brain regions with less FC than in the healthy control group; T value > 0 , brain regions with more FC than in the healthy control group. FC: Functional connectivity; MNI: Montreal Neurological Institute; RRMS: relapsing-remitting multiple sclerosis.

Table 3 | Brain regions in which the FC with the left and right side of the brain in the right hippocampus differed between the RRMS and healthy control groups

Brain region (automated anatomical labeling template)	Voxel (mm ³)	MNI coordination			T value
		X	Y	Z	
Left side					
Temporal lobe (inferior temporal gyrus, middle temporal gyrus), hippocampal uncus, limbic lobe	7	-21	-3	-42	4.930
Middle temporal gyrus, superior temporal gyrus	12	-45	0	-33	-5.117
Frontal lobe	9	0	33	-27	-5.281
Temporal lobe, fusiform gyrus	5	-48	-18	-27	5.785
Limbic lobe, hippocampal uncus, para-hippocampal gyrus	11	-9	-3	-21	-9.253
Occipital lobe (inferior occipital lobe, medial occipital lobe), fusiform gyrus, posterior cerebellar lobe	76	-27	-75	-18	7.879
Insula, frontal lobe	29	-42	-3	0	8.138
Medial frontal lobe, limbic lobe, anterior cingulate gyrus	56	-3	60	3	-6.545
Frontal lobe, temporal lobe (superior temporal gyrus), precentral gyrus	12	-54	3	9	5.274
Occipital lobe, occipital lobe, middle temporal gyrus	15	-24	-87	9	5.451
Parietal lobe, precuneus	98	-15	-60	48	8.056
Parietal lobe (inferior parietal lobule), postcentral gyrus, frontal lobe	147	-39	-48	39	7.619
Frontal lobe, paracentral lobule	32	0	-33	69	7.176
Right side					
Posterior cerebellar lobe	21	18	-72	-42	6.304
Posterior cerebellar lobe, cerebellar tonsil, right brain stem	60	6	-45	-45	-7.962
Fusiform gyrus, temporal lobe, occipital lobe, anterior cerebellar lobe	38	42	-48	-27	7.231
Temporal lobe (superior temporal gyrus)	5	45	18	-27	-6.073
Temporal lobe (middle temporal gyrus)	27	63	-45	-6	6.119
Midbrain	10	3	-15	-15	-7.379
Frontal lobe, insula	58	30	24	-6	6.433
Frontal lobe (anterior medial frontal gyrus, anterior inferior frontal gyrus)	184	-39	39	0	7.437
Occipital lobe	17	33	-81	12	5.657
Frontal lobe (anterior medial frontal gyrus)	130	42	39	15	6.985
Insula, parietal lobe	6	42	-21	21	5.066
Caudate nucleus, frontal lobe	12	18	15	21	-6.595
Temporal lobe (middle temporal gyrus), angular gyrus, parietal lobe	22	48	-66	24	6.248
Prefrontal lobe, precentral gyrus	6	60	-6	24	4.479
Precuneus, parietal lobe	48	21	-57	48	6.948
Frontal lobe (medial frontal lobe), cingulate gyrus, limbic lobe	44	6	27	45	8.231
Frontal lobe, precentral gyrus, postcentral gyrus	37	24	-27	69	4.955

RRMS patients (corrected $P < 0.001$ using AlphaSim voxel > 5). T value < 0 , brain regions with less FC than in the healthy control group; T value > 0 , brain regions with more FC than in the healthy control group. FC: Functional connectivity; MNI: Montreal Neurological Institute; RRMS: relapsing-remitting multiple sclerosis.

Differences in ReHo between the groups

Compared with the HC group, the ReHo values were decreased in the left hippocampus, limbic lobe, and para-hippocampal gyrus of the RRMS patients, whereas the ReHo values were increased in the partial hippocampus, temporal lobe, limbic lobe, and para-hippocampal gyrus (Table 4 and Figure 3). Compared with the HC group, ReHo values were significantly higher in the right para-hippocampal gyrus, limbic lobe, hippocampus, and temporal lobe in the RRMS group ($P < 0.05$, corrected by AlphaSim, voxel > 5 ; Table 4 and Figure 3).

Table 4 | Brain regions in which the ReHo values in the left and right hippocampus differed between the patients in the RRMS group and the healthy control group

Brain region (automated anatomical labeling template)	Voxel (mm ³)	MNI coordination			T value
		X	Y	Z	
Left side					
Hippocampus, limbic lobe, para-hippocampal gyrus	6	-21	-15	-21	-3.604
Hippocampus, temporal lobe, limbic lobe, para-hippocampal gyrus	13	-33	-27	-15	3.705
Right side					
Para-hippocampal gyrus, limbic lobe, hippocampus, temporal lobe	9	33	-15	-21	3.219

RRMS patients (corrected $P < 0.05$ using AlphaSim voxel > 5). T value < 0 , brain regions with less ReHo than in the healthy control group; T value > 0 , brain regions with more ReHo than in the healthy control group. MNI: Montreal Neurological Institute; ReHo: regional homogeneity; RRMS: relapsing-remitting multiple sclerosis.

Differences in ALFF between the groups

Compared with the HC group, when the left hippocampus was used as the template, the ALFF values of patients in the RRMS group were lower in the left hippocampus, limbic lobe, para-hippocampal gyrus, temporal lobe, and caudate nucleus. The ALFF values of the following brain regions were higher in the RRMS group than in the HC group: the limbic lobe, para-hippocampal gyrus, hippocampus, and amygdala (Table 5 and Figure 4). When the right hippocampus was used as the template, the ALFF values of patients in the RRMS group were lower than those in the HC group in the hippocampus, limbic lobe, temporal lobe, para-hippocampal gyrus, and amygdala; and the ALFF values were higher in the hippocampus, para-hippocampal gyrus, and limbic lobe (Table 5 and Figure 4).

Table 5 | Brain regions in which the ALFF values in the left and right hippocampus differed between the patients in the RRMS and healthy control groups

Brain region (automated anatomical labeling template)	Voxel (mm ³)	MNI coordination			T value
		X	Y	Z	
Left side					
Limbic lobe, para-hippocampal gyrus, hippocampus, amygdala	17	-24	-21	-15	7.384
Hippocampus, limbic lobe, para-hippocampal gyrus, temporal lobe, caudate nucleus	49	-36	-27	-9	-5.329
Right side					
Hippocampus, limbic lobe, temporal lobe, para-hippocampal gyrus, amygdala	36	36	-12	-24	-7.822
Hippocampus, para-hippocampal gyrus, limbic lobe	10	24	-21	-15	4.154

RRMS patients (corrected $P < 0.05$ using AlphaSim voxel > 5). T value < 0 , brain regions with less ALFF than in the healthy control group; T value > 0 , brain regions with more ALFF than in the healthy control group. ALFF: Amplitude of low-frequency fluctuation; MNI: Montreal Neurological Institute; RRMS: relapsing-remitting multiple sclerosis.

Differences in hippocampal and whole-brain FA and MD between the groups

Compared with the HC group, the FA values were lower in the RRMS group in the bilateral hippocampus ($P < 0.05$, corrected by FDR, voxel > 10 ; Table 6). The MD values in the left hippocampus were significantly higher in the RRMS group than in the HC group ($P < 0.05$, corrected by FDR, voxel > 10 ; Table 6).

Table 6 | Comparison of FA and MD values in the bilateral hippocampi between the RRMS and healthy control groups

DTI parameter	RRMS group (n = 20)	Healthy control group (n = 16)	T value	P value
FA value of left hippocampus	0.373±0.016	0.397±0.015	-2.374	0.008 [†]
FA value of right hippocampus	0.336±0.021	0.379±0.025	-4.635	0.023 [†]
MD value of left hippocampus	0.167±0.052	0.034±0.012	3.430	0.003 [†]
MD value of right hippocampus	0.079±0.025	0.041±0.015	-1.416	0.200

Data are expressed as the mean ± standard deviation, [†] $P < 0.05$, vs. healthy control group. Corrected by FDR, voxel > 10 . DTI: Diffusion tensor imaging; FA: fractional anisotropy; MD: mean diffusivity; RRMS: relapsing-remitting multiple sclerosis.

Correlation between clinical characteristics and functional and structural changes in RRMS patients

We conducted correlation analyses to examine the relationships between the left and right resting-state fMRI measurements (including the FC, ReHo, and ALFF values) and the MoCA and EDSS scores in the RRMS group. The results indicated that the whole-brain FC was negatively correlated with the MoCA score in the left hippocampus ($r = -0.698$, $P = 0.025$), and that whole-brain FC was negatively correlated with EDSS score in the right hippocampus ($r = -0.649$, $P = 0.042$). The MD value was negatively correlated with the MoCA score ($r = -0.729$, $P = 0.017$) and positively correlated with the EDSS score ($r = 0.653$, $P = 0.041$) in the left hippocampus. Furthermore, the MD value was positively correlated with the EDSS score in the right hippocampus ($r = 0.684$, $P = 0.029$). The ReHo value, ALFF value, and FA value were not statistically significantly correlated with the EDSS or MoCA scores ($P > 0.05$). The ALFF value in the right hippocampus was positively correlated with the right FA value ($r = 0.693$, $P = 0.026$), and the ReHo value in the right hippocampus was negatively correlated with the right FA value ($r = -0.709$, $P = 0.022$; Table 7 and Figure 5).

Table 7 | Correlation analysis between hippocampal function/structure and MoCA and EDSS scores in RRMS patients

DTI parameter	MoCA score		EDSS score	
	r	P	r	P
zFCmap of left hippocampus	-0.698	0.025	/	/
zFCmap of right hippocampus	/	/	-0.649	0.042
MD value of left hippocampus	-0.729	0.017	0.653	0.041
MD value of right hippocampus	/	/	0.684	0.029

We used the Pearson correlation coefficient. The left hippocampus whole-brain FC was negatively correlated with the MoCA score. The whole-brain FC of the right hippocampus was negatively correlated with the EDSS score. The MD value of the left hippocampus was negatively correlated with the MoCA score and positively correlated with the EDSS score. The right hippocampal MD value was positively correlated with the EDSS score. DTI: Diffusion tensor imaging; EDSS: extended disability status scale; FC: functional connectivity; MD: mean diffusivity; MoCA: Montreal cognitive assessment; RRMS: relapsing-remitting multiple sclerosis.

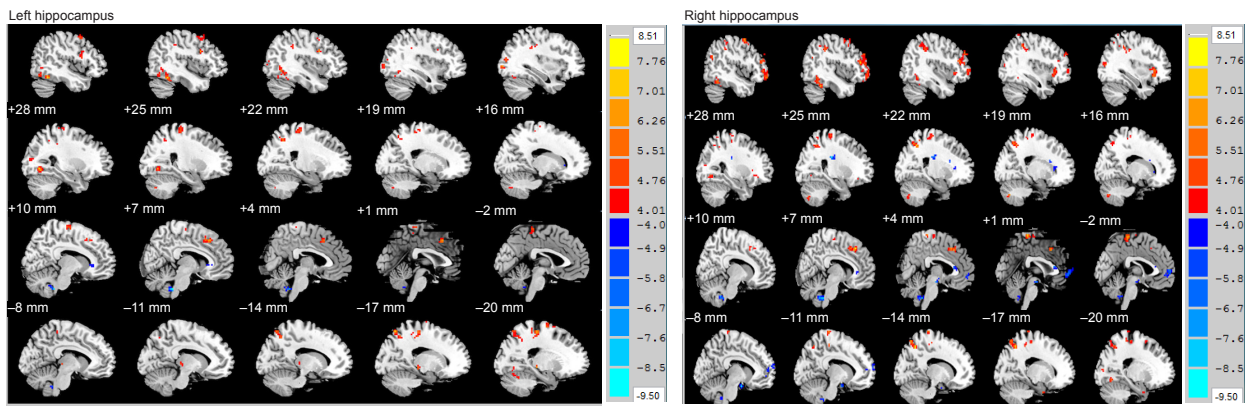


Figure 2 | Whole-brain FC analysis of the bilateral hippocampus in relapsing-remitting multiple sclerosis patients and healthy control subjects.

The left hippocampal regions with attenuated FC on the left and right side of the brain were the cingulate gyrus, limbic lobe, and right brain stem; while the regions with enhanced whole brain FC were the right cerebellum (posterior cerebellum), bilateral temporal lobe, suboccipital lobules, frontal lobe (prefrontal lobe, frontal gyrus), amygdala, lateral globus pallidus, and lentiform nucleus. The right hippocampal regions with attenuated FC on the left and right side of the brain were the right brain stem, limbic lobe, middle temporal gyrus, para-hippocampal gyrus, and left frontal lobe; while the regions with enhanced whole-brain FC were the right cerebellum (posterior cerebellum), left occipital lobe, left frontal lobe, insula, and parietal lobe. Red represents FC-enhanced regions and blue represents FC-attenuated regions. Independent two-sample *t*-tests were performed for the standardized FC ($P < 0.001$, corrected by AlphaSim, voxel > 5). Ordinate unit: *T*-value. FC: Functional connectivity.

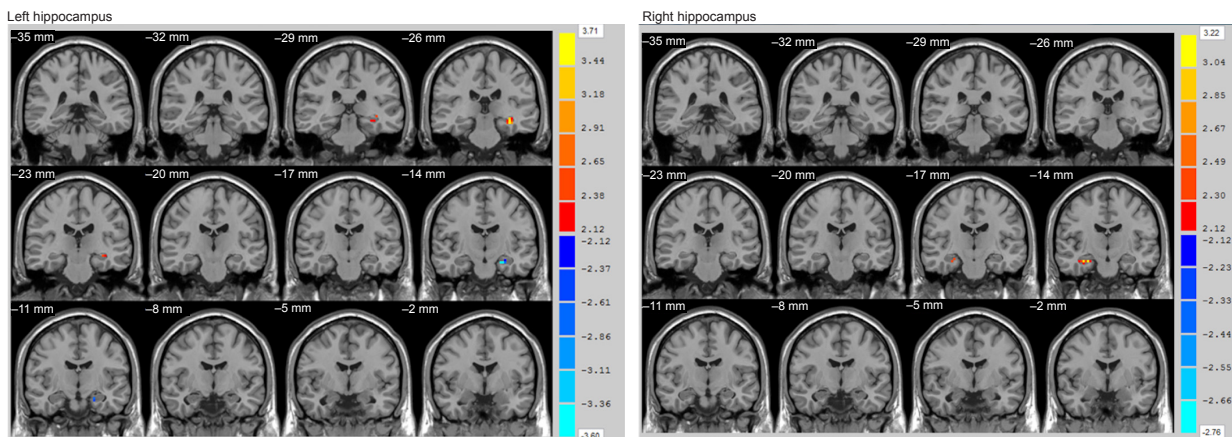


Figure 3 | ReHo analysis of the bilateral hippocampus in relapsing-remitting multiple sclerosis patients and healthy control subjects.

The ReHo values of the left hippocampus, limbic lobe, and para-hippocampal gyrus were decreased in the patients compared with the control subjects, whereas the ReHo values of the partial hippocampus, temporal lobe, limbic lobe, and para-hippocampal gyrus were increased in the relapsing-remitting multiple sclerosis patient group compared with the healthy control group. The right para-hippocampal gyrus, limbic lobe, hippocampus, and temporal lobe had significantly higher ReHo values in the relapsing-remitting multiple sclerosis group compared with the healthy control group. Red represents an ReHo increase and blue represents an ReHo decrease. Independent two-sample *t*-tests were performed for the standardized ReHo maps ($P < 0.05$, corrected by AlphaSim, voxel > 5). Ordinate unit: *T*-value. ReHo: Regional homogeneity.

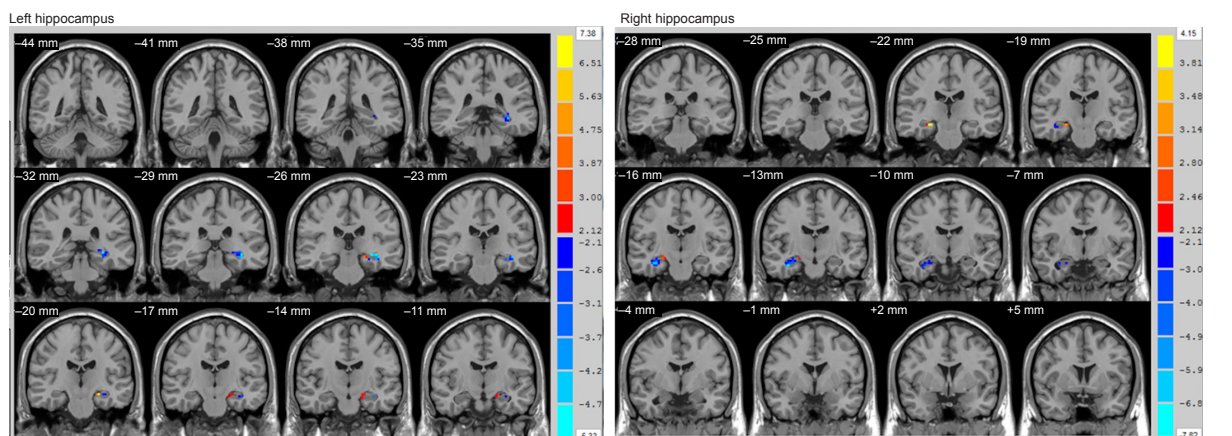


Figure 4 | ALFF analysis of the bilateral hippocampus of relapsing-remitting multiple sclerosis patients and healthy control subjects.

Compared with the healthy control group, ALFF values in the relapsing-remitting multiple sclerosis group were lower in the left hippocampus, limbic lobe, para-hippocampal gyrus, temporal lobe, and caudate nucleus. The ALFF values of the following brain regions were higher in the relapsing-remitting multiple sclerosis group than in the healthy control group: limbic lobe, para-hippocampal gyrus, hippocampus, and amygdala. Red represents the ALFF increase; blue represents ALFF decrease. Independent two-sample *t*-tests were performed for the standardized ALFF maps ($P < 0.05$, corrected by AlphaSim, voxel > 5). Ordinate unit: *T*-value. ALFF: Amplitude of low-frequency fluctuation.

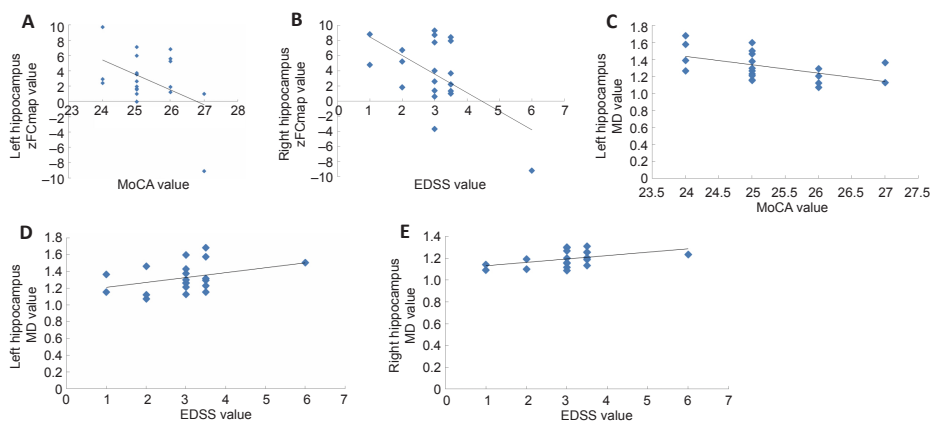


Figure 5 | Correlation between the hippocampal FC or MD value and clinical scores (Pearson correlation coefficient).

(A) The correlation between the FC of the left hippocampus and the MoCA score ($r = -0.698$, $P = 0.025$). (B) The correlation between the FC of the right hippocampus and the EDSS score ($r = -0.649$, $P = 0.042$). (C) The correlation between the MD value of the left hippocampus and the MoCA score ($r = -0.729$, $P = 0.017$). (D) The correlation between the MD value of the left hippocampus and the EDSS score ($r = 0.653$, $P = 0.041$). (E) The correlation between the MD value of the right hippocampus and the EDSS score ($r = 0.684$, $P = 0.029$). EDSS: Extended disability status scale; FC: functional connectivity; MD: mean diffusivity; MoCA: Montreal cognitive assessment.

Discussion

In this study, we used structural and functional MRI to investigate how cognitive impairment was associated with hippocampal structure and connectivity in RRMS patients. Our data indicated that the whole brain and local FC of the hippocampus in patients with RRMS were both enhanced and weakened, such that abnormal FC of the left hippocampus was related to cognitive function, while abnormal FC of the right hippocampus was related to the extent of disability.

Although MS is defined as the presence of white matter lesions in the central nervous system, it can also involve changes in gray matter, including cortical atrophy and damage observed via MRI and histology (Kuceyeski et al., 2018; Yamout and Alroughani, 2018). Subcortical atrophy in the thalamus (Amin and Ontaneda, 2020; Zhao et al., 2020) and hippocampal atrophy (van Geest et al., 2018; Lashkari et al., 2021) are also associated with cognitive deficits (Karavasilis et al., 2019). fMRI can be used to effectively assess neural connectivity and damage in various brain regions (Filippi and Rocca, 2013). DTI is a non-invasive imaging technique, and the only method capable of illustrating the structure and orientation of white matter fibrous structures *in vivo*. Thus, it allows for the evaluation of changes in the brain microstructure (Pokryszko-Dragan et al., 2018; Schneider et al., 2019; Martínez-Heras et al., 2020). Martínez-Heras et al. (2020) demonstrated that MS lesions could be classified into two types based on the severity of changes in macroscopic DTI parameters and microscopic diffusion properties.

Based on the distribution of resting-state FC patterns of the left and right hippocampi in patients in the RRMS and HC groups, the FC patterns of the bilateral hippocampi, cerebral cortex, and subcortical regions showed enhanced and attenuated regions in RRMS patients. The abnormal regions were mainly distributed in the prefrontal lobe, parietal lobe, temporal lobe, limbic lobe, and cerebellum regions. The correlation between cognitive dysfunction and the left hippocampus was more significant than that for the right hippocampus ($r = -0.698$), which was consistent with previous studies (Sicotte et al., 2008; Hulst et al., 2012). Attenuated connectivity indicates impaired FC. The enhanced connectivity was due to cerebral cortical reorganization in adjacent areas, which is a compensatory mechanism for maintaining neurological stability that is highly common in RRMS patients (Filippi et al., 2013; Sacco et al., 2013; Sampath et al., 2017; Laura et al., 2018). In this study, the amygdala, lateral globus pallidus, and lentiform nucleus exhibited enhanced FC patterns. Given the presence of compensatory mechanisms, we predicted that those brain regions would be abnormal in patients with cognitive impairment. fMRI of cognitively

impaired patients revealed a functional relationship between the posterior cingulate gyrus and the hippocampus. The activation of the posterior cingulate gyrus during episodic memory coding was related to the activation of the right hippocampus. The identification of memory was related to the activation of the left hippocampus, indicating the presence of hemispherical characteristics (Klawiter et al., 2011).

The ReHo value represents brain functional activity based on the BOLD signal, which can be interpreted as a network-centric indicator. This underscores the importance of connectivity nodes in the human brain via local functional interactions (Jiang and Zuo, 2016). In the present study, we found that RRMS patients exhibited abnormal ReHo values in the limbic lobe, para-hippocampal gyrus, and temporal lobe. These brain regions are mainly involved in cognitive-related activities. Indeed, the changes in ReHo values in these regions were related to cognitive impairment. In the left hippocampus, the ALFF values of the hippocampus, limbic lobe, para-hippocampal gyrus, temporal lobe, and caudate nucleus were decreased in the RRMS patient group compared with the HCs. The ALFF values of the hippocampus, limbic lobe, temporal lobe, para-hippocampal gyrus, and amygdala were also lower in the right hippocampus in the RRMS group. These findings suggest that these brain regions may be highly susceptible to cognitive impairment caused by immunological white matter lesions. The spontaneous changes in ALFF values in these brain regions are a potential indicator of MS nerve damage. In behavioral studies, MS patients exhibited significant cognitive, memory, and executive function deficits, with abnormal information processing speed (Defrancesco et al., 2013; Li et al., 2017; Koenig et al., 2019; Toko et al., 2021). The hippocampus is a key structure in multiple memory functions, including memory coding and retrieval, and is also strongly associated with learning and emotional control. Previous studies have reported that the hippocampus is particularly sensitive to ischemia and hypovolemia (Fein et al., 2000; Chai et al., 2010; Yang et al., 2010). Thus, a decrease in ALFF values in the hippocampus may indicate memory impairment in subjects with MS. The association between altered caudate nucleus activity and reduced cognitive function has not been reported in previous fMRI studies.

We identified an interesting phenomenon in the current study: the FC of the hippocampus was both enhanced and attenuated, in different regions. This could be related to changes in connectivity caused by the spontaneous activities of neurons in other brain regions. We postulated that this was the result of compensatory mechanisms in these brain regions. Indeed, the hippocampus is not a monolithic structure (Heine et al., 2020). Recently, a study on hippocampal connectivity

precisely defined the pre- and post-differentiation regions of the hippocampus. The phenomenon observed in the present study could have been caused by variation between the distinctive histological regions of the hippocampus. This topic merits in-depth examination in future research.

In this study, our voxel-based analysis revealed that bilateral hippocampal myelination (MD value) and the loss of white matter integrity (FA value) in patients in the RRMS group were significantly different from those in the HC group. Because of the presence of a small amount of fibrous bundles in the gray matter, axonal injury may occur in the hippocampus of patients with RRMS, and neuronal cells may be retrogradely damaged, resulting in cell degeneration, apoptosis, necrosis, increased cell junction, and reduced diffusion of water molecules. These form the pathological basis for FA and MD abnormalities (Feinstein et al., 2010), and lead to shrinkage in the corresponding gray matter volume. Previous studies have used voxel-based morphological analyses to identify reduced hippocampal volume in RRMS patients (Han et al., 2017). Hippocampal atrophy in MS is caused by neuronal damage, and fibrous bundle demyelination is also evident (Papadopoulos et al., 2009). Recent studies have reported abnormal changes in FA values or MD values in cortical or subcortical lesions in RRMS patients (Cicarelli et al., 2001; Ceccarelli et al., 2007; Preziosa et al., 2011). Although MD values are usually increased, FA values may either increase (Cicarelli et al., 2001; Tovar-Moll et al., 2009) or decrease (Ceccarelli et al., 2007). These inconsistent changes could be explained by grey matter inflammation, where the resting state or activation of microglia during grey matter inflammation could lead to an increase or decrease in FA values, respectively (Calabrese et al., 2011). In this study, FA values in both hippocampal regions decreased, indicating that this brain region was inflamed. The FA value of the left hippocampus was greater than that of the right hippocampus because the FA value was positively correlated with the voxel size (Han et al., 2017). We postulate that the volume shrinkage of the right hippocampus was more obvious than that of the left in this study, which is consistent with previous results (Roosendaal et al., 2010).

We assessed structural and functional changes in hippocampal memory networks and their association with cognitive dysfunction in RRMS patients. Our study demonstrates that whole-brain FC was abnormal in the bilateral hippocampi, but only abnormal left hippocampal connectivity was associated with cognitive function. The lateralization and asynchrony of hippocampal damage observed in RRMS patients may indicate that bilateral hippocampal damage occurs in a different time frame, although more evidence is needed on this topic (Roosendaal et al., 2010). The results from this study reveal that the whole-brain FC of the right hippocampus was negatively correlated with the EDSS score. Previous studies have suggested that gray matter atrophy is associated with white matter fiber damage (Koubiyr et al., 2018). We postulate that the hippocampus directly or indirectly affects the degree of disability in patients with RRMS through white matter fiber damage. A reduction in hippocampal volume is associated with impairment of general knowledge and contextual visual memory (Resmini et al., 2012). Fjalldal et al. (2018) reported that changes in white matter integrity in the right hippocampus were associated with decreased visual spatial capacity, whereas decreased white matter integrity and demyelination/edema in the left hippocampus were associated with impaired general knowledge and delayed recall in episodic memory. Here, we report a correlation between functional and structural changes in the hippocampus. The changes in the ALFF value, ReHo value, and ipsilateral FA

value in the right hippocampus were particularly robust. We thus conclude that local hippocampal functional changes are associated with fibrous bundle damage. Previous studies have reported that gray matter atrophy was strongly correlated with the FA values in white matter fibrous bundles (Han et al., 2017). We postulate that local hippocampal functional changes are associated with hippocampal volume shrinkage and white matter fiber damage. There are numerous causes for the decreases in cognitive ability associated with changes in hippocampal function and volume. Previous studies have reported that a slight decrease in FC could occur in patients with normal hippocampal volume, suggesting that FC changes can occur before hippocampal volume atrophy takes place (Roosendaal et al., 2010). Longitudinal studies are needed to clarify how FC changes are related to atrophy in hippocampal memory systems.

Improvements in the reliability and repeatability of functional connectivity models will enhance the utility of this approach (Penner and Aktas, 2017). Currently, it is difficult to determine whether individual changes are normal compensatory phenomena (Rocca and Filippi, 2017) or pathological changes. Therefore, to examine differences in FC in the brain, the underlying disease mechanisms must be combined with the characteristics of the disease (Abrol et al., 2017). Alterations in brain FC is expected to provide potential biomarkers (Du et al., 2010) for classifying or predicting brain disorders, enabling diagnoses at the early stage of disease.

There are some limitations to this study. First, this study was a preliminary cross-sectional study. Future longitudinal studies are warranted to investigate the reproducibility of the FC and DTI abnormalities in the hippocampus of MS patients, and longitudinal studies are necessary to clarify how FC changes are related to hippocampal memory system atrophy. Second, this study had a small sample size (20 subjects). Further studies with a larger sample size and comprehensive clinical data are warranted to explore the correlations between FC, structural abnormalities in the hippocampus, and clinical metrics.

In conclusion, the FC and structural abnormalities of the hippocampus are correlated with the degree of cognitive function and extent of disability in RRMS patients.

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