Chapter 3

Hippocampal damage in multiple sclerosis: Detection and functional effects
3.3

Structural and functional hippocampal changes in multiple sclerosis patients with intact memory

Stefan D Roosendaal
Hanneke E Hulst
Heleen EM Feenstra
Hugo Vrenken
Jonas A Castelijns
Petra JW Pouwels
Frederik Barkhof
Jeroen JG Geurts

Radiology, in press
ABSTRACT

**Purpose:** To investigate changes in hippocampal functional connectivity and structural measures of hippocampal damage in multiple sclerosis (MS) patients with intact spatial memory, a cognitive domain frequently affected in progressive MS.

**Materials and Methods:** The study protocol was approved by the institutional ethics review board and all subjects gave written informed consent prior to participation. Twenty-five MS patients with intact spatial memory function were compared to 30 age- and sex-matched controls. Hippocampal volume differences, based on manually drawn masks, were evaluated using the Student's t-test. Additionally, focal hippocampal lesions and mean diffusivity were obtained as descriptive measures of structural hippocampal damage. Multiple regression analyses of the resting state functional magnetic resonance imaging (fMRI) data were performed for each subject using hippocampal time series. Between-group analyses were conducted with a mixed effects model, corrected for multiple comparisons by a cluster defining threshold of $Z=2$ and a corrected cluster size significance level of $p<0.05$.

**Results:** Right hippocampal was significantly lower in MS patients as compared to controls ($p<0.01$). Left hippocampal volume was also less in MS patients compared to controls, but not significantly so ($p=0.09$). Resting state functional connectivity between the hippocampus and its anatomical input or target areas, among which the anterior cingulate gyrus, thalamus and prefrontal cortex, were significantly decreased in MS patients. Decreased hippocampal functional connectivity was more pronounced in a subgroup of MS patients with hippocampal atrophy, although subtle decreases of functional connectivity were also found in patients with normal hippocampal volume.

**Conclusion:** In MS patients, significant abnormalities of hippocampal functional connectivity are already present before spatial memory function is impaired, especially in those patients with more pronounced hippocampal atrophy. Longitudinal studies should now assess whether these functional connectivity and structural changes may precede memory impairment in MS.
INTRODUCTION

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system, which is commonly diagnosed in the prime of life and in most cases leads to chronic disability. In addition to classical inflammatory white matter (WM) lesions, grey matter (GM) demyelination is common and extensive, as shown in previous, histopathological studies. [1-3] Abnormalities in the GM are also consistently found with in vivo magnetic resonance imaging (MRI) [4-6] and are correlated with clinical deficits. [7,8]

In addition to motor and sensory deficits, cognitive decline is reported in up to 65% of patients suffering from MS. [9,10] Besides impaired information processing speed and working memory performance, deficits in spatial memory are commonly found. [9,11] The hippocampus is of critical importance to spatial memory function [12] and part of the observed memory impairment in MS is likely to be caused by damage to this structure.

Evidence for hippocampal demyelination in MS has been reported in recent histopathological studies. [13,14] In vivo MRI studies showed the existence of focal hippocampal hyperintensities [15] and hippocampal atrophy. [16] How hippocampal damage affects hippocampal function and memory performance, however, is not well established.

A promising method to investigate functional connectivity of the brain is resting state functional MRI (fMRI). In studies investigating resting state fMRI, subjects are instructed to keep their eyes closed or fixated at a cross-hair during an imaging acquisition that commonly takes ten minutes, and to avoid falling asleep. Spontaneous low frequency fluctuations of the cerebral blood oxygenation level-dependent (BOLD) signal can then be measured [17] and are thought to represent the baseline activity of the brain, providing consistent information about functional connectivity between brain regions. [18,19] The resting state fMRI data can be analyzed using a seed region-of-interest (ROI) when a hypothesis is available, resulting in functional connectivity maps; conversely, resting state fMRI can be analyzed without an a priori hypothesis in a data-driven manner, for example, with independent component analysis (ICA).

At present, it is not clear at what time point and to what extent spatial memory is affected by hippocampal damage in MS. Traditional imaging
approaches may not be sufficient in understanding the complex dynamic system in which the hippocampus is involved. Studying functional connectivity of the hippocampus with resting state fMRI and relating it to structural integrity measures may be of value. In general, the dissociation between structural damage and memory function may be more pronounced in earlier stages of the disease. We hypothesize that MRI measures of hippocampal functional connectivity may already be affected when patients still have intact memory. The aims of the current study were therefore to investigate changes in hippocampal functional connectivity and structural measures of hippocampal damage in multiple sclerosis (MS) patients with intact spatial memory, a cognitive domain frequently affected in progressive MS.

**MATERIALS AND METHODS**

**Subjects**
The study protocol was approved by the institutional ethics review board and all subjects gave written informed consent prior to participation. For patients, inclusion criteria included either a confirmed diagnosis of MS or a high suspicion of MS after a first clinical event. The exclusion criteria for all subjects in this study were presence or history of psychiatric or neurological disease (for patients: other than MS), claustrophobia, inability to minimize movement during scanning session, presence of contra-indications for MRI, or treatment with corticosteroids in the month before participation. Thirty patients were prospectively recruited from a clinical MS database and all fulfilled the abovementioned criteria. From these patients, 25 had an intact spatial memory (defined in the next paragraph), and data of these patients were analyzed for this study. Mean age of these patients was 38.9 (± 8.3) years (female patients: 39.3 ± 8.7 years; male patients: 38.1 ± 8.0 years) and 17 of them were female. Five patients had a clinically isolated syndrome (CIS), 18 patients had a relapsing-remitting (RR) disease type and two patients were in the secondary progressive (SP) phase of the disease. [20] The median expanded disability status scale (EDSS) [21] was 3.0 (interquartile range [IQR]: 2.0 – 4.0), mean disease duration was 4.5 (± 3.8) years. Thirty age- and sex-matched healthy controls (mean age 40.5 ± 10.1 years; female controls: 41.5 ± 10.5 years; male
controls 38.3 ± 9.4 years; 20 females) were also included, all of them fulfilled the inclusion- and exclusion criteria. Our first subject was investigated on 28th of November 2006, the last subject was investigated on the 31st of July 2007.

**Neuropsychological assessment**
All subjects underwent a neuropsychological examination (S.D.R., two years of experience in neuropsychological testing) on the day of scanning. Spatial memory was assessed, as we were specifically interested in hippocampal function, using the Location Learning Test (LLT). [22] The learning phase consists of five consecutive trials and generates a total displacement score called the LLT total score, where a score of zero indicates perfect performance and scores increase with more errors. Thirty minutes after the learning phase a sudden delayed recall trial was performed, which generated a measure for ‘rapid forgetting’, the LLT delay score. Intact spatial memory in patients was defined as an LLT total score of ≤ 1 SD above the mean score of controls and a (near-) perfect LLT delay score lower than five, indicating absence of rapid forgetting.

Symptoms indicative of depression and anxiety, which could bias memory function, were assessed by the Hospital Anxiety and Depression Scale (HADS-A and HADS-D). [23] Fatigue was assessed by the Checklist of Individual Strength (CIS-20) questionnaire. [24] Pre-morbid intelligence was measured using the Dutch version of the New Adult Reading Test (DART). [25,26] Lastly, handedness was evaluated in all subjects using the Edinburgh Handedness Scale (EHS). [27]

**Magnetic resonance imaging**
MR imaging was performed on a 1.5T whole-body MR system (Siemens Sonata, Erlangen, Germany), using an eight-channel phased-array head coil. For resting state functional MR imaging, 200 volumes of echo planar images (EPI) were acquired (TR 2850 ms, TE 60 ms; 36 axial slices with 3.3x3.3x3.3 mm³ isotropic resolution; acquisition time 9.5 minutes). Subjects were instructed to rest with their eyes closed, not fall asleep, and think of nothing in particular during this scan. Single-slab T1-weighted magnetization prepared rapid acquisition gradient-echo (MPRAGE) images (TR 2700 ms, TE 5 ms, TI 950 ms; 1.3x1.3x1.3 mm³ isotropic resolution) were obtained. Furthermore,
diffusion-weighted EPI (DTI; TR 8500 ms, TE 86 ms; 2x2x2 mm$^3$ isotropic resolution) were acquired, with 60 volumes with non-collinear diffusion gradients (b-value of 700 s mm$^{-2}$) and ten volumes without directional weighting. Single slab three-dimensional double inversion recovery (3D-DIR) images (TR 6500 ms, TE 355 ms, TI 350 / 2350 ms; 1.3 mm thickness and 1.2x1.2 mm$^2$ in-plane resolution) were acquired to assess cortical and hippocampal lesions. Lastly, interleaved turbo spin-echo proton density- (PD) and T2-weighted images (TR 3130 ms, TE 24 / 85 ms; 46 3-mm-thick axial slices and 1.0x1.0 mm$^2$ in-plane resolution) were obtained to assess white matter lesions.

Brain volumes, hippocampal volume and diffusivity measures, and lesion measurements

All image manipulation tools used in this study, except the AFNI tool used for temporal filtering of fMRI data, are part of the FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl), and all of these tools were operated by S.D.R, with three years experience in digital image analysis. Brain volume normalized for head size (NBV) was measured on the MPRAGE images using SIENAX. Furthermore, hippocampal masks were drawn (H.E.M.F., with one year experience in outlining the hippocampus), according to a standard operating procedure based on previous reports. [28] All hippocampal volumes provided in this paper were corrected for subject head size.

In addition to the primary marker of atrophy as an indicator of structural hippocampal damage, hippocampal mean diffusivity was also investigated. Motion and eddy current distortion of the diffusion-weighted images were corrected with FDT, which was also used to fit the diffusion tensor and derive mean diffusivity (MD) for each voxel. The hippocampal masks were co-registered to the diffusion images with FLIRT using mutual information as the cost function. The co-registered hippocampal masks were kept conservative by intensity thresholding at a value of 0.25. Subsequently, mean left and mean right hippocampal MD was extracted for each subject.

WM, hippocampal and cortical lesions were marked and used as descriptive measures of focal damage. Hyperintense WM lesions were marked and manually outlined (H.E.H., two years of experience in MR image analysis in MS) on the PD images using in-house-developed software with a local-threshold technique; subsequently, the WM lesion volume was calculated for
each patient. Hippocampal and cortical lesions were scored (S.D.R., three years of experience in 3D-DIR image analysis in MS) in a similar fashion to previous studies. [15,29]

**Functional MRI**

For each subject, the full MRI protocol could be acquired, and there were therefore no differences between individuals in the number of functional MR volumes (i.e. 200 data points). The functional MR images were motion corrected and non-brain tissue was removed with BET. Subsequently, the functional MR images were band-pass filtered using AFNI [30] between 0.01 and 0.08 Hz, to exclude higher frequency physiological and lower frequency scanner drift-related confounds. [31]

The hippocampal masks were co-registered to the functional MR images with FLIRT. The co-registered hippocampal masks were kept conservative by intensity thresholding at a value of 0.5 (which is higher than the threshold value used for DTI, because of a larger voxel size of the functional MR images). After this, the mean and linear trend of the band-pass filtered images were removed and left and right hippocampal time series of each subject were extracted.

The band-pass filtered images were spatially smoothed in FEAT (FWHM: 5 mm), and grand-mean scaling was applied (mean-based intensity normalization of all volumes by the same factor) in order to allow comparisons between data sets at a group level.

**Statistical analyses**

Multiple regression analyses of the fMRI data were performed for each subject using FEAT. [32] A general linear model was created for each hippocampal timeseries, which produced individual statistical connectivity maps for the left and right hippocampus. All individual statistical maps were linearly co-registered to the MNI152 standard space. Group-level analyses, controlling for age and gender, were conducted with FLAME [33] using a mixed-effects model. Within-group maps of voxels significantly connected with each hippocampus were corrected for multiple comparisons by a cluster defining threshold of $Z=4$ and a corrected cluster size significance level of $p<0.05$. Similarly, between-group contrast maps were corrected by applying a threshold of $Z=2$ and a corrected cluster size significance level of $p<0.05$. 
Synchronization of hippocampal activity was calculated using the correlation coefficient between left and right hippocampal time series. The correlation coefficient has previously been used in MS in magnetoencephalography (MEG) [34] and fMRI [35] studies as a simple measurement of synchronization between homologous areas of both hemispheres.

All other statistical analyses in this study were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA). Student’s t-test was used when data was normally distributed, otherwise the Mann-Whitney U test was used. Values are reported as mean ± standard deviation (SD), unless indicated otherwise. P values <0.05 were considered statistically significant.

Table 1 | Measures of controls and multiple sclerosis patients without memory impairment.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Controls (n=30)</th>
<th>Patients (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized hippocampal volume (NHV), left (mL)</td>
<td>4.48 ± 0.48</td>
<td>4.24 ± 0.54</td>
<td>.09</td>
</tr>
<tr>
<td>NHV, right (mL)</td>
<td>4.72 ± 0.39</td>
<td>4.35 ± 0.52</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Mean diffusivity (MD), left (x 10^-3 mm^2 s^-1)</td>
<td>1.00 ± 0.05</td>
<td>1.04 ± 0.06</td>
<td>.01</td>
</tr>
<tr>
<td>MD, right (x 10^-3 mm^2 s^-1)</td>
<td>1.01 ± 0.05</td>
<td>1.04 ± 0.06</td>
<td>.03</td>
</tr>
<tr>
<td>Normalized brain volume (NBV; L)</td>
<td>1.62 ± 0.06</td>
<td>1.60 ± 0.08</td>
<td>.20</td>
</tr>
<tr>
<td>Synchronization of hippocampal activation</td>
<td>0.50 ± 0.15</td>
<td>0.43 ± 0.18</td>
<td>.16</td>
</tr>
<tr>
<td>Location Learning Test (LLT) total score†</td>
<td>12 (7-24)</td>
<td>10 (4-19)</td>
<td>.26</td>
</tr>
</tbody>
</table>

Data are means ± standard deviation, except for † where because of non-normal distribution median and interquartile range are provided.

RESULTS

Subject descriptives
Median T2 lesion load of our MS patients was relatively low, specifically 1.8 (IQR: 0.8 - 8.1) mL. The mean IQ of controls (106 ± 10), calculated from the DART, was comparable to that of patients (107 ± 13). Based on the Edinburgh Handedness Scale, 26 controls and 23 patients were right-handed, two controls and one patient were left-handed, and two controls and one patient were ambidextrous. Median HADS-A and HADS-D scores of controls (1 and 4)
were comparable to those of patients (4 and 5, respectively). HADS scores for depression and for anxiety can range from 0 to 21; all patient scores were lower than 11, below which existence of depression or anxiety disorder is unlikely. [36] The CIS-20 fatigue score can range from 20 to 140, in patients the median fatigue score was 54 (IQR: 39 - 83) and in controls 37 (IQR: 22 - 50).

**Hippocampal volume and diffusivity measures, and brain volumes**
In controls, right-sided normalized hippocampal volume was higher than left-sided NHV (Table 1). The MS patients were found to have a significantly lower right NHV than controls (p<0.01). Left NHV was also lower in patients than controls, but not significantly so (p=0.09). No significant difference between right and left NHV was found in patients. Normalized brain volume (NBV) did not differ significantly between MS patients and controls.

Mean diffusivity (MD) was significantly higher in patients than in controls in both the left and right hippocampus. Mean NHV (average of left and right) was correlated with hippocampal MD neither in controls (r=0.1; p=0.6), nor in patients (r=0.04; p=0.9).

**Functional connectivity of the hippocampi with other brain areas**
In healthy controls, areas functionally connected to the left hippocampus were the right hippocampus, cerebellum, bilateral anterolateral temporal lobe, bilateral anterior and posterior cingulate cortex, left and right thalamus, left and right caudate nucleus, bilateral medial prefrontal and medial parietal cortex (Figure 1A). Functional connectivity of the left hippocampus in the total MS patient group showed a similar pattern to that of controls (Figure 1B), but was significantly decreased in patients with the left cerebellum, bilateral anterior insular cortex, left and right thalamus, left and right caudate nucleus and the anterior cingulate cortex (Figure 1C).

Functional connectivity of the right hippocampus in healthy controls, shown in Figure 2A, exhibited functional patterns similar to those of the left hippocampus. Functional connectivity of the right hippocampus in our patients (Figure 2B) was found to be significantly decreased with the left amygdala and hippocampus, as well as with the left insular cortex, compared to controls (Figure 2C).
Figure 1 | Resting state functional MRI (fMRI) group connectivity maps of the left hippocampus with the rest of the brain for controls and patients. Background image is the group mean magnetization prepared rapid acquisition gradient-echo (MPRAGE) image in standard space. **A**: The left hippocampus is connected in controls with the right hippocampus, cerebellum, left and right insular cortex, cingulate gyrus, mammillary bodies, and medial prefrontal and parietal cortex. **B**: Patients show the same pattern of left hippocampal connectivity as controls, albeit less strong and smaller. **C**: Statistical difference map showing that in the patient group the left hippocampus is significantly less functionally connected with the left cerebellum, left and right anterior insular cortex, left and right thalamus, left and right caudate nucleus and anterior cingulate cortex. No areas were found where connectivity was significantly stronger in patients.

To investigate whether functional connectivity changes were associated with structural hippocampal damage, analyses of functional connectivity and synchronization of hippocampal activity were also performed for two patient subgroups separately, defined by the presence or absence of hippocampal atrophy. For this purpose, patients with a mean NHV lower than one SD below
the control group mean NHV were considered to have hippocampal atrophy. Fourteen out of the total of 25 patients (56%) were found to have normal mean hippocampal size, whereas the remaining 11 patients (44%) had hippocampal atrophy.

Patients with normal hippocampal size did show areas of decreased functional connectivity of the left and right hippocampus when compared to controls as well as higher functional connectivity when compared to patients with decreased hippocampal size; however these differences were only visible at sub-threshold values, and not at our threshold of significance of $Z=2$.

In patients with hippocampal atrophy as compared to controls, functional connectivity of the left hippocampus was diminished with the right hippocampus, left amygdala, bilateral medial prefrontal cortex, left thalamus, bilateral medial prefrontal cortex, bilateral insular cortex, bilateral posteroventral cingulate gyrus and bilateral anterior cingulate cortex (Figure 3A). Also in patients with hippocampal atrophy as compared to controls, functional connectivity of the right hippocampus was diminished with the left cerebellum, the tail of the left hippocampus, and the left insular cortex (Figure 3B). Our analyses did not yield any areas of significantly increased functional connectivity of the left or right hippocampus in the total MS patient group, or the subgroups, compared to controls.

No significant difference was found between patients with hippocampal atrophy and patients with normal hippocampal size in hippocampal lesion number (Table 2), a measure of focal hippocampal damage. Figure 4 shows examples of 3D-DIR images of normal control hippocampus and of a hippocampal lesion in a patient. Also, cortical lesion number did not differ significantly between the two patient subgroups. In Figure 5 an example of a cortical lesion on 3D-DIR is shown. No hippocampal or cortical lesions were found in healthy controls.

**Synchronization of hippocampal activity**
Synchronization of hippocampal activity, measured as the cross-correlation of left and right hippocampal time series, of our total MS group did not differ significantly from that of controls. However, when our otherwise homogeneous MS group (Table 2) was divided according to normal or decreased hippocampal size, synchronization of hippocampal activity of patients with normal
hippocampal size was comparable to that of controls, whereas synchronization of hippocampal activity of patients with hippocampal atrophy was significantly decreased compared to that of patients with normal hippocampal size and to that of controls.

Table 2 | Measures of patients with normal hippocampal size (NHS) compared to patients with hippocampal atrophy (HA)

<table>
<thead>
<tr>
<th>Measure</th>
<th>NHS (n=14)</th>
<th>HA (n=11)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normalized hippocampal volume (NHV), left (mL)</td>
<td>4.63 ± 0.30</td>
<td>3.75 ± 0.33</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>NHV, right (mL)</td>
<td>4.67 ± 0.37</td>
<td>3.94 ± 0.37</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Mean diffusivity (MD), left (x 10^-3 mm^2 s^-1)</td>
<td>1.05 ± 0.07</td>
<td>1.04 ± 0.05</td>
<td>.73</td>
</tr>
<tr>
<td>MD, right (x 10^-3 mm^2 s^-1)</td>
<td>1.04 ± 0.06</td>
<td>1.04 ± 0.05</td>
<td>.77</td>
</tr>
<tr>
<td>Normalized brain volume (NBV; L)</td>
<td>1.61 ± 0.07</td>
<td>1.59 ± 0.08</td>
<td>.49</td>
</tr>
<tr>
<td>Synchronization of hippocampal activation</td>
<td>0.50 ± 0.14</td>
<td>0.35 ± 0.18</td>
<td>.03</td>
</tr>
<tr>
<td>Age (years)</td>
<td>38.1 ± 8.6</td>
<td>39.9 ± 8.3</td>
<td>.59</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>4.1 ± 3.9</td>
<td>5.0 ± 3.8</td>
<td>.56</td>
</tr>
<tr>
<td>Disease type (clinically isolated syndrome [CIS] / relapsing remitting [RR] / secondary progressive [SP])</td>
<td>4/9/1</td>
<td>1/9/1</td>
<td></td>
</tr>
<tr>
<td>Expanded disability status scale (EDSS)</td>
<td>2.8 (2.0-3.6)</td>
<td>3.0 (2.5-4.0)</td>
<td>.61</td>
</tr>
<tr>
<td>T2 lesion volume (mL)</td>
<td>1.3 (0.6-5.8)</td>
<td>4.7 (1.7-10.6)</td>
<td>.09</td>
</tr>
<tr>
<td>Hippocampal lesion number</td>
<td>0 (0-1.3)</td>
<td>0 (0-1.0)</td>
<td>.94</td>
</tr>
<tr>
<td>Cortical lesion number</td>
<td>3.0 (0.8-7.3)</td>
<td>8.0 (3.0-11.0)</td>
<td>.11</td>
</tr>
</tbody>
</table>

Above the line, data are means ± standard deviation, below the line median (interquartile range) are provided.

**DISCUSSION**

Our study has shown that functional connectivity between the hippocampus and several cortical areas is significantly decreased in MS patients who otherwise have minor brain atrophy, a low T2 lesion load and, most notably, intact spatial memory. This decreased functional connectivity of the hippocampus was driven by a subgroup of patients with hippocampal atrophy and synchronization of hippocampal activity in this group was found to be significantly reduced compared to that of patients with normal hippocampal
size. More subtle decreases of functional connectivity compared to controls were found in patients with normal hippocampal size.

Impaired memory has a high impact on quality of life and, since it is frequently found in MS, [9,37] it is likely that part of our patient group will develop impairment of memory in the future. Learning and consolidation of spatial memory in particular depend heavily on the hippocampus. [12,38] The LLT is one of the few standardized object-location memory tasks that has been validated in patient groups and was shown to be sensitive enough for detecting subtle spatial memory deficits. [39] False-positive test results are unlikely because the LLT does not require precise motor function, verbal responses or complex instructions. Thus, by using the LLT we have applied one of the most robust tests available to exclude prevalence of memory dysfunction specifically relying on the hippocampus in our patients. Other forms of memory impairment were not available in our study and it can thus not be ruled out that these may have been present in some of our patients.

Direct evidence for hippocampal involvement in MS comes from numerous previous publications. Hippocampal demyelination in MS has been shown histopathologically. [13,14] In vivo, focal hippocampal lesion numbers were similar to those reported in the histopathological studies. [15] Hippocampal abnormalities in MS patients have not been limited to demyelination and atrophy, since hippocampal gliosis was suggested by in vivo MR spectroscopy findings [40] and reduced hippocampal glucose metabolism was found using PET. [41] Additionally, the fornix was found to be damaged in a recent whole-brain DTI study. [42] The current study adds to these results by showing decreased connectivity of the hippocampi, which may exist even before hippocampal atrophy becomes apparent.

The hippocampi of our patients showed signs of lateralized damage: right hippocampal volume was affected more than left hippocampal volume, and functional connectivity was conversely decreased with more target or input areas for the left hippocampus as compared the right one. In our controls, as well as in controls from several other studies, [43,44] right hippocampal volumes were consistently found to be higher than left hippocampal volumes. Studies in rats [45] and in humans [46] report right dominance for spatial memory. The lateralization of hippocampal damage in our patients most likely indicates that in MS left and right hippocampal damage occur in different time frames, but
more research is needed to provide definitive answers. Although hippocampal MD was significantly increased in patients, it was not related to hippocampal volume, possibly reflecting different pathological processes.

Figure 2 | Resting state functional MRI (fMRI) group connectivity maps of the right hippocampus with the rest of the brain for controls and patients. Again, the background image is the group mean magnetization prepared rapid acquisition gradient-echo (MPRAGE) image in standard space. A: The right hippocampus is connected in controls with the left hippocampus and, similarly to the left hippocampus, to the cerebellum, left and right insular cortex, cingulate gyrus, mammilary bodies, and medial prefrontal and parietal cortex. B: In patients, the right hippocampus is connected to the same areas as in controls. C: Statistical difference map showing that in the patient group the right hippocampus is significantly less functionally connected with the left hippocampus and the left anterior insular cortex. No areas were found where connectivity was significantly stronger in patients.
**Figure 3** | Statistical difference maps showing areas of lower connectivity with the left (A) and right (B) hippocampus in the group of patients with hippocampal atrophy compared to the control group. 

A: In the group of patients with hippocampal atrophy the left hippocampus has significantly less functional connectivity with the left cerebellum, left and right anterior insular cortex, medial prefrontal cortex, and anterior cingulate cortex. 

B: In the group of patients with hippocampal atrophy, the right hippocampus is significantly less functionally connected with the left cerebellum, left hippocampus, and left insular cortex.

Hippocampal volumes, obtained in our study by manual outlining shown to be more accurate than automatic hippocampal segmentation, [47] were corrected for head size. Because control and patient (sub)groups were age- and
sex-matched, and no evidence for depression and anxiety disorder was found, influence of other variables known to affect hippocampal volume was minimal.

![Figure 4](image)

**Figure 4** | Coronal three-dimensional double inversion-recovery (3D-DIR) images showing normal hippocampi in a 31 year-old male control (left image) and a hippocampal lesion depicted by a thick arrow in a 45 year-old female multiple sclerosis (MS) patient (right image). (Juxta)cortical lesions and white matter (WM) lesions can also be seen in this patient.

Exchange of information between the hippocampus and cortical areas for the purpose of cortical long-term memory formation is known to occur during rest and sleep. [38] The specific use of resting state fMRI allowed us in the current study to investigate this process, by measuring spontaneous low-frequency fluctuations, that are thought to reflect intrinsic activity of the brain. [48] Interestingly, neocortical areas known to project to and from the hippocampus [49] were indeed found to be functionally connected with both left and right hippocampus in our controls. Decreased functional hippocampal connectivity in our patients during rest can be interpreted according to the same framework: the communication process between the hippocampi and the cortex that provides information input to the hippocampus and enhances long-term memory is disturbed. Decreased connectivity was associated with hippocampal damage, as the largest connectivity decreases were found in the group of patients with hippocampal atrophy (compared with controls). Although not reaching statistical significance, more subtle decreases of
functional connectivity were also found in patients with normal hippocampal sizes compared to controls, possibly indicating that functional connectivity changes may predate hippocampal atrophy. Future (longitudinal) studies are necessary to further clarify how functional connectivity changes and atrophy of the hippocampal memory system are related temporally.

Figure 5 | Axial three-dimensional double inversion-recovery (3D-DIR) images demonstrating normal cortical grey matter (GM) in a 31 year-old male control (left image) and a cortical GM lesion depicted by an arrowhead in a 26 year-old female MS patient (right image).

Interhemispheric synchronization, calculated with the correlation coefficient, was found to be decreased in MS both during rest and a finger-tapping fMRI task. [35] Interestingly, synchronization between homologue brain areas was predominantly decreased between the temporal regions in MS in a MEG study. [50] There is recent evidence that spontaneous correlation patterns may be similar to functional networks in task-fMRI, [51] and that they relate to task performance. [52] The ability of synchronization of hippocampal activity to distinguish between patient groups should still prove its value in other MS settings.

Task-fMRI studies in MS have so far not evaluated specific hippocampal memory function but mainly working memory, during which increased
hippocampal activation was found in CDMS patients compared to CIS patients [53] and increased temporal (and prefrontal) activation in MS patients with good recall compared to those with impaired recall. [54]

Our study suffered from several limitations. We could not exclude that hippocampal time series signals were to some extent biased by partial volume of cerebrospinal fluid signal. This bias was made less likely by the fact that the co-registered hippocampal masks were shrunken after registration with a strict threshold of 0.5. With respect to resting state fMRI, another possible limitation is the contribution of respiratory and cardiac pulsations to between-subject differences. [55,56] By applying temporal filtering however, respiratory and cardiac induced signal variations can be largely removed from signal fluctuations of interest. Any remaining noise may decrease the sensitivity for detection of between-group differences, but false-positive results will only occur when there is a difference in physiological noise between groups, which is not expected in our study. Resting state studies have received the criticism that resting state correlation patterns may merely reflect uncontrolled mental tasks. [57] However, recent studies show that spontaneous BOLD correlation patterns are similar across different behavioural states, such as different resting states, sleep, and anaesthesia. [48] Thus, the BOLD correlation patterns seem to rather represent an intrinsic property of the brain. As a last possible limitation of our study, exogenous corticosteroids are known to alter resting state fMRI fluctuations. [58] Six patients in our study, evenly distributed over the two patient subgroups, received intravenous methylprednisolon treatment during their disease course, but none of them received it in the month preceding participation in our study.

In conclusion, our study showed that in MS patients with intact spatial memory, widespread and interhemispheric decreased hippocampal functional connectivity can be found. Results of our study suggest that functional connectivity changes may even predate hippocampal atrophy. Future longitudinal studies should further clarify the temporal paths of functional connectivity decreases and hippocampal atrophy, and should investigate whether these changes are predictive for impaired memory in MS patients.
REFERENCES


57. Stark CE, Squire LR. When zero is not zero: the problem of ambiguous baseline conditions in fMRI. Proc Natl Acad Sci U S A 2001; 98(22):12760-12766.