Introduction

Memory deficits develop in 40–65% of the patients with multiple sclerosis (MS) and can lead to problems in daily life activities and to unemployment.1–3 The hippocampus plays an essential role in memory function and is therefore a crucial structure to study to understand the underlying neurobiological substrates of memory impairment in MS.

Previous studies showed widespread structural damage of the hippocampus in MS, such as extensive demyelination4–6 and volume loss7,8 which were all related to cognitive decline. Our earlier work also showed changes in hippocampal activation in MS patients using functional magnetic resonance imaging (fMRI) during a memory task. Increased brain activation was detected in the (para)hippocampal areas in cognitively preserved MS patients, while decreased activation was observed in the same regions in cognitively impaired patients.9

It is unclear whether these changes in hippocampal activation also involve changes in functional connectivity of the hippocampus. Previously, a decrease in

Multiple Sclerosis Journal
2015, Vol. 21(13) 1705–1712
DOI: 10.1177/1352458514567727
© The Author(s), 2015.
Reprints and permissions: http://www.sagepub.co.uk/journalsPermissions.nav

Original Research Paper

Memory impairment in multiple sclerosis: Relevance of hippocampal activation and hippocampal connectivity

Hanneke E Hulst, Menno M Schoonheim, Quinten Van Geest, Bernard MJ Uitdehaag, Frederik Barkhof and Jeroen JG Geurts

Abstract

Background: Memory impairment is frequent in multiple sclerosis (MS), but it is unclear what functional brain changes underlie this cognitive deterioration.

Objective: To investigate functional hippocampal activation and connectivity, in relation to memory performance in MS.

Methods: Structural and functional magnetic resonance imaging data were acquired for 57 MS patients and 28 healthy controls (HCs), yielding hippocampal measures of volume, lesions, functional activation during a memory task and functional connectivity at rest. Memory function was based on two subtests of a larger neuropsychological test battery and related to hippocampal neuroimaging measures, using linear regression.

Results: Hippocampal volume was lower in MS patients, as compared to HCs. In MS, hippocampal activation during the task was increased in cognitively preserved, but decreased in cognitively impaired, patients. Increased hippocampal connectivity was detected in MS patients, as compared to HCs, between the left hippocampus and the right posterior cingulate. Memory impairment in MS was explained (adjusted $R^2 = 0.27$) by male gender, decreased hippocampal activation and increased hippocampal connectivity ($p = 0.001$).

Conclusions: Decreased activation of the hippocampus, increased connectivity and male gender were associated with worse memory performance in MS. These results indicate that increased activation and increased connectivity do not always coincide, and relate differently to cognitive dysfunction in MS.

Keywords: cognitive dysfunction, memory, hippocampus, hippocampal activation, hippocampal connectivity, magnetic resonance imaging, multiple sclerosis

Date received: 6 July 2014; accepted: 17 December 2014
hippocampal connectivity with the anterior cingulate and prefrontal cortices, as measured with resting-state fMRI, was found in MS patients with intact memory function\textsuperscript{10}; however, another study linked increased connectivity of the default mode network, which usually includes the hippocampus, to cognitive impairment in MS.\textsuperscript{11}

Based on these previous pathological and imaging results, we now aimed to investigate this structure in even more depth, by studying the interplay between hippocampal activation and hippocampal connectivity in MS patients. We used a combination of specific hippocampal measures: task-induced activation, resting-state connectivity, volume and lesions. Our ultimate goal was to examine the relative importance of these hippocampal measures in a comprehensive model, to define the most important hippocampal measure(s) associated with memory function in MS.

**Methods**

**Participants**

Table 1 summarizes all demographics. A total of 85 subjects were enrolled as part of a previous study,\textsuperscript{9} including 57 patients with MS (mean age 47.6; 39 women) and 28 healthy controls (HCs; mean age 44.6; 19 women) that were matched for age, gender and educational level. Patients were diagnosed with clinically-definite MS following the revised McDonald criteria,\textsuperscript{12} with a mean disease duration of 11.3 years. Patients either had a relapsing–remitting (n = 40) or secondary progressive (n = 17) MS disease course.

Physical disability was measured using a telephone version of the Expanded Disability Status Scale (EDSS).\textsuperscript{13} Patients were relapse-free and without steroid treatment for at least 6 weeks. The institutional ethics review board approved the study and all subjects gave written informed consent prior to participation.

**Neuropsychological examination**

All subjects underwent an extensive neuropsychological test battery, specifically designed to assess memory function. Hulst et al.\textsuperscript{9} has a detailed description of the tests. In short, the following tests were included:

1. Verbal learning and memory task (VLGT), the Dutch equivalent of the California Verbal Learning Test\textsuperscript{14};
2. Location Learning Test (LLT), for visuospatial memory\textsuperscript{15};
3. Digit span (working memory, both forward and backward: subtests of the Wechsler Adult Intelligence Scale);\textsuperscript{16}
4. Semantic word fluency test (word list generation (WLG), knowledge, access to semantic

| Table 1. Demographic, clinical and radiological data of patients with MS and HCs. |
|-----------------------------------------------|-----------------|-----------------|-------|
| Age (yrs)                                    | 47.58 (7.62; 26.93 – 60.57) | 44.63 (8.66; 28.25 – 60.48) | 0.113 |
| Gender (female/male)                         | 39/18           | 19/9            | 0.958 |
| Disease subtype                              | RRMS: 40       | –               | –     |
| Disease duration (yrs)                       | 11.34 (6.74; 1.00 – 31.00) | –               | –     |
| Educational level\textsuperscript{a}         | 6.00 (5.00 – 6.00) | 6.00 (5.00 – 6.00) | 0.818 |
| EDSS\textsuperscript{a}                      | 4.00 (3.50 – 5.00) | –               | –     |
| HADS Anxiety\textsuperscript{a}             | 5.00 (4.00 – 8.00) | 3.00 (2.00 – 6.75) | 0.010 |
| HADS Depression\textsuperscript{a}          | 4.00 (2.50 – 7.50) | 1.00 (0.00 – 3.75) | < 0.001 |
| HADS Checklist of individual strength\textsuperscript{a} | 75.00 (53.00 – 90.00) | 26.50 (16.25 – 51.25) | < 0.001 |
| Normalized brain volume (L)                  | 1.40 (0.08; 1.24 – 1.57) | 1.47 (0.07; 1.35 – 1.63) | < 0.001 |
| Normalized gray matter volume (L)            | 0.74 (0.05; 0.64 – 0.83) | 0.78 (0.05; 0.87 – 0.78) | < 0.007 |
| Normalized white matter volume (L)           | 0.66 (0.04; 0.57 – 0.76) | 0.70 (0.04; 0.63 – 0.77) | < 0.001 |
| T1 lesion volume (mL)\textsuperscript{a}     | 2.04 (0.81 – 4.86) | –               | –     |
| T2 lesion volume (mL)\textsuperscript{a}     | 5.62 (2.98 – 10.36) | –               | –     |

\textsuperscript{a}Non-normally distributed variables for which median and interquartile range are presented.

EDSS: Expanded Disability Status Scale; HC: healthy controls; L: liters; mL: milliliter; MS: multiple sclerosis; RRMS: relapsing–remitting MS; SPMS: secondary progressive MS; yrs: years. HADS: Hospital Anxiety and Depression Scale.
memory and long-term verbal memory)\textsuperscript{17,18}; and

5. Information processing speed (Letter Digit Substitution Test (LDST)).\textsuperscript{19}

**Composite memory score**

To specifically investigate hippocampus-related cognitive function, the test scores on the VLGT and LLT were combined into a composite memory score. For all tests separately, a Z-score was calculated using the following formula:

$$Z = \frac{\text{Individual test score} - \text{mean score of the controls}}{\text{SD of the controls}}$$

The Z-scores of the two subtests were then added for each subject.

**Confounders of cognitive functioning**

Symptoms of depression, anxiety and fatigue were assessed using the Hospital Anxiety and Depression Scale (HADS)\textsuperscript{20} and the Checklist of Individual Strength (CIS-20).\textsuperscript{21}

**Magnetic resonance imaging**

Imaging was performed using a 1.5T Siemens Sonata scanner. Lesion-based sequences included two-dimensional (2D) proton-density/T2-weighted fast spin-echo (with a repetition time (TR) of 3130 ms, echo time (TE) of 24/85 ms, 46 contiguous 3-mm axial slices and in-plane resolution of $1 \times 1$ mm$^2$), 2D spin-echo T1-weighted imaging (TR 485 ms, TE 12.0 ms, 46 contiguous 3-mm axial slices and in-plane resolution of $1 \times 1$ mm$^2$) and three-dimensional (3D) double-inversion recovery images (TR of 2350 ms, TE of 355 ms, inversion time (TI) of 350 ms, $1.2 \times 1.2 \times 1.2$ mm$^3$ voxel size and 120 sagittal slices).

Brain volumes and hippocampal volumes were determined using a 3D-T1 magnetization-prepared rapid acquisition gradient-echo (TR of 2700 ms, TE of 5.0 ms, TI of 950 ms, 1.3 mm isotropic voxel size and 176 sagittal slices).

Hippocampal function was measured during task-based fMRI, using an episodic memory-encoding paradigm (echo planar imaging (EPI) of 208 volumes, TR of 2220 ms, TE of 60 ms, 28 axial slices of 3 mm that included the hippocampal area and $3.3 \times 3.3$ mm$^3$ voxel size), as well as during resting-state (i.e. eyes closed, no task; EPI covering the entire brain using 200 volumes, TR of 2850 ms, TE of 60 ms, 36 contiguous axial slices and $3.3 \times 3.3 \times 3.3$ mm$^3$ voxel size). Resting-state fMRI was always performed prior to the task-based fMRI.

**Lesion volume and brain volumes**

We measured T2-hyperintense and T1-hypointense lesion volumes using a local thresholding technique. Whole-brain volumes were analyzed using SIENAX (part of FMRIB Software Library (FSL) 5; www.fmrib.ox.ac.uk/fsl), providing normalized whole-brain (NBV), gray matter (NGMV) and white matter (NWMV) volumes.

**Structural hippocampal measures**

Hippocampal volume was estimated using FIRST\textsuperscript{22} (part of FSL), normalized using the V-scaling factor from SIENAX. Hippocampal lesions were counted on the Double Inversion Recovery (DIR) images by an experienced rater (author HE Hulst).

**Functional hippocampal measures: Activation**

The episodic memory paradigm consisted of an encoding and retrieval phase. During encoding, 50 different novel landscape images were presented to the subjects; then 30 minutes following encoding, the retrieval phase was initiated. Here, a total of 100 landscape images were shown, 50 of which were novel and another 50 that were already presented during the encoding phase. Subjects had to indicate whether they had seen the picture before or not. Task-based activation was calculated for the correctly remembered items (as measured during the retrieval) from the encoding phase, using FSL’s ‘FEAT’. This was based on the assumption that when someone remembered a landscape picture correctly, the encoding had been successful. To specifically investigate activation differences within the hippocampus, we calculated the average Z-values in the left and right hippocampus during the successful encoding of landscape images for every subject, separately (details on the paradigm are in Hulst et al.\textsuperscript{9}; the resting-state fMRI data was not previously published).

**Functional hippocampal measures: Connectivity**

Resting-state fMRI pre-processing used standard FSL protocols, including motion correction, smoothing and high-pass filtering (100-second cut-off); resting-state data were kept in subject space. We assessed functional connectivity between both the hippocampi and the rest of the brain, using an atlas that was derived from a combination of the cortical regions of the Automated Anatomical Labeling (AAL) atlas (part of MRIcro; www.cabiatl.com/mricro/micro/
template.html) and the subcortical regions of FIRST (part of FSL, performed on the 3D T1 sequence). The AAL atlas was registered to each subject’s 3D T1 scan by inverting the nonlinear standard space registration parameters derived from FNIRT (also part of FSL), and masked with individual gray matter masks derived from SIENAX. The complete atlas was co-registered to the subject’s resting-state scan, using an inverted, boundary-based registration matrix (BBR; part of FSL5). This resulted in an individualized atlas in subject space, featuring 92 regions of interest covering all the gray matter. Average time series were created for each region of interest; and functional connectivity was calculated between each hippocampus and every other region of interest of the brain, using synchronization likelihood (SL) in BrainWave (http://home.kpn.nl/stam7883/brainwave.html). SL is a measure for linear and non-linear synchronization between brain regions that ranges from zero to one, previously applied to fMRI data to assess functional connectivity in MS23 and Alzheimer’s disease.24

Statistical analysis: MS patients versus HCs
All variables were checked for normality using Kolmogorov-Smirnov testing and histogram inspection. To achieve normality, the SL values were inverted. All analyses were corrected for age, gender and educational level. We used multivariate General Linear Model analyses to assess group differences and effect sizes are mentioned in partial $\eta^2$. We only included those connectivity variables reaching $p < 0.01$ in the Linear Regression Model below.

Relationship with memory performance
To find the most important hippocampal predictor(s) of memory performance, a backward linear regression model was fitted with the following predictors: hippocampal volume, hippocampal lesion count, hippocampal task-induced functional activation and hippocampal functional connectivity. Age, gender and education level were included as covariates. $P$-values of 0.05 were considered significant.

Results
Demographic and clinical data are presented in Table 1. Between-group comparisons revealed no differences in age, gender and educational level. As expected, patients scored higher on anxiety ($p = 0.010$) and depression ($p < 0.001$, both measured on the HADS); and on fatigue (CIS-20; $p < 0.001$), than the HCs. In Table 1, we present the lesion load and brain volumes. NBV was lower in patients with MS, as compared to HCs ($\eta^2 = 0.17; p < 0.001$), as well as NGMV (partial $\eta^2 = 0.09; p < 0.007$) and NWMV (partial $\eta^2 = 0.16; p < 0.001$).

Neuropsychology
Neuropsychological test scores are provided in Table 2. Patients with MS had lower test scores on all tests, except for the digit span forward ($p = 0.145$) and WLG category professions ($p = 0.066$), compared to controls. The composite memory score was worse in patients than in HCs (mean patient $Z = −2.64; p < 0.001$). We found 20 patients were impaired (i.e. Z-score of $−2$ or lower) on one memory test and 8 patients were impaired on both tests. Impairment was most frequently seen on the LLT (23 patients), followed by the VLGT (13 patients).

Neuroimaging of the hippocampus
Hippocampal volume and hippocampal lesions.
Compared to HCs, patients with MS had a lower normalized hippocampal volume (left hippocampus: partial $\eta^2 = 0.136; p = 0.007$ and right hippocampus: partial $\eta^2 = 0.122; p = 0.002$ (Table 3)). The median number of hippocampal lesions was 1 (range 0–2.75) in the patient group.

Task-related hippocampal activation. In the entire patient group, the average fMRI signal in the hippocampus during the encoding of successfully recalled items was similar to HCs (left hippocampus: $p = 0.739$ and right hippocampus: $p = 0.195$), for both hippocampi (Table 3); however, in our previous analysis on the same patient group, we subdivided the MS patients into cognitively preserved and cognitively impaired patients, based on the whole neuropsychological test battery (Z-scores of at least $−2$, on two out of the five tests). The results showed that hippocampal activation was increased in the cognitively preserved and reduced in the cognitively impaired patients.9

Resting-state functional connectivity of the hippocampus.
Patients showed increased functional connectivity of both hippocampi with several brain regions, compared to HCs (Table 3). The left hippocampus showed increased connectivity with the right posterior cingulate ($p = 0.002$), left thalamus ($p = 0.028$) and left inferior temporal lobe ($p = 0.031$). The right hippocampus showed increased connectivity with the right caudate nucleus ($p = 0.023$), left Heschl’s gyrus ($p = 0.029$), left anterior cingulate ($p = 0.034$), left amygdala ($p = 0.040$) and left nucleus accumbens ($p = 0.048$).
Only the functional connection between the left hippocampus and the right posterior cingulate was significant, at $p < 0.01$, and was therefore included as a predictor in the regression analysis (hereafter referred to as the functional connectivity of the left hippocampus).

### Predicting memory performance in MS

Regression analyses were conducted to predict the memory performance in MS patients and HCs separately. Candidate predictors included in the model were: functional connectivity of the left hippocampus, task-related signal changes of the left and right hippocampus separately, volume of the left and right hippocampus separately, total number of hippocampal lesions, age, gender and educational level.

In patients, memory performance could be explained (adjusted $R^2 = 0.27; F = 6.33; p = 0.001$) by male gender (standardized $\beta = -0.363; p = 0.009$), decreased task-related activation of the right hippocampus (standardized $\beta = 0.366; p = 0.009$) and increased functional connectivity of the left hippocampus (standardized $\beta = 0.277; p = 0.043$). In controls, the same model (adjusted $R^2 = 0.22; F = 7.21; p = 0.013$), indicating gender as the only predictor (standardized $\beta = -0.473$).

Although increased left hippocampal connectivity and decreased right hippocampal activation were both related to memory performance, these two variables were not significantly correlated to each other ($r = -0.24; p = 0.11$).

### Discussion

Earlier studies showed that both structural and functional hippocampal abnormalities were related to memory impairment in MS; however, it is not clear how functional activation and connectivity changes of the hippocampus relate to each other, in the context of memory impairment. In the current study, we used a comprehensive combination of hippocampal functional activation and connectivity to investigate presumed underlying mechanism(s) related to memory impairment, in patients with MS. We found that decreased functional hippocampal activation and increased functional hippocampal connectivity, together, explain a large percentage of memory impairment in MS. These two functional modalities predicted memory performance in different ways and explained more of the memory impairment than structural hippocampal measures.

Hippocampal activation in response to a specific task (i.e. memory encoding) is a local measure of hippocampal function; whereas functional connectivity is a more global measure that provides information on how well the hippocampus ‘communicates’ with other regions of the brain at rest. Decreased activation of the right hippocampus and increased functional connectivity of the left hippocampus with the right posterior cingulate were associated with impaired memory function in MS. Hippocampal activation and

---

### Table 2. Neuropsychological test scores of patients with MS and HCs (mean (SD; min – max)).

<table>
<thead>
<tr>
<th>Measure</th>
<th>MS ($n=57$)</th>
<th>HCs ($n=28$)</th>
<th>$F$-value</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verbal learning and memory task$^a$</td>
<td>55.00 (42.00 – 61.50)</td>
<td>65.50 (53.50 – 69.00)</td>
<td>15.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total number of correct items</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total number of substitutions</td>
<td>49.25 (11.96; – 12.00 – 73.00)</td>
<td>64.14 (9.54; 40.00 – 84.00)</td>
<td>29.69</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Location learning test$^a$</td>
<td>21.00 (10.00 – 33.00)</td>
<td>7.50 (3.00 – 13.00)</td>
<td>11.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Total number of displacements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digit span</td>
<td>8.00 (7.00 – 10.00)</td>
<td>10.00 (8.00 – 11.75)</td>
<td>2.17</td>
<td>0.145</td>
</tr>
<tr>
<td>Backward$^a$</td>
<td>6.00 (4.00 – 8.00)</td>
<td>7.00 (6.25 – 8.00)</td>
<td>9.22</td>
<td>0.003</td>
</tr>
<tr>
<td>Word list generation: Animals</td>
<td>21.70 (5.75; 9.00 – 35.00)</td>
<td>25.29 (7.25; 9.00 – 42.00)</td>
<td>5.89</td>
<td>0.018</td>
</tr>
<tr>
<td>Word list generation: Professions</td>
<td>16.35 (5.31; 6.00 – 33.00)</td>
<td>18.29 (5.38; 7.00 – 28.00)</td>
<td>3.48</td>
<td>0.066</td>
</tr>
<tr>
<td>Word list generation: M-words$^a$</td>
<td>9.00 (5.50 – 11.00)</td>
<td>10.00 (7.0 – 14.00)</td>
<td>5.62</td>
<td>0.020</td>
</tr>
<tr>
<td>Memory performance Z-score$^a$</td>
<td>$-2.06 (− 3.77 – (− 0.12))$</td>
<td>$0.32 (− 0.91 – 1.39)$</td>
<td>$c$</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

$^a$Non-normally distributed variables in which median and interquartile range are presented.

$^b$All test scores were converted into Z-scores (relative to HCs) and subsequently tested for group differences.

$^c$Indicates Mann–Whitney U testing.

HCs: healthy controls; MS: multiple sclerosis.
Multiple Sclerosis Journal 21(13)

Table 3. Hippocampus-specific imaging measures in patients with MS and HC’s.

<table>
<thead>
<tr>
<th>Measure</th>
<th>MS (n = 57) mean</th>
<th>HC (n = 28) mean</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(SD; min – max)</td>
<td>(SD; min – max)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Structural measures</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left hippocampal volume (mL)</td>
<td>4.848 (0.778; 1.756 – 6.509)</td>
<td>5.462 (0.548; 4.438 – 6.346)</td>
<td>12.317</td>
<td>0.001</td>
</tr>
<tr>
<td>Right hippocampal volume (mL)</td>
<td>4.754 (1.061; 1.583 – 6.966)</td>
<td>5.456 (0.495; 4.415 – 6.460)</td>
<td>10.803</td>
<td>0.002</td>
</tr>
<tr>
<td>Number of hippocampal lesionsa</td>
<td>1.000 (0.000 – 2.750)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Task-related activation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left hippocampal activation</td>
<td>0.431 (0.771; −1.291 – 2.091)</td>
<td>0.510 (0.640; −0.473 – 1.927)</td>
<td>0.112</td>
<td>0.739</td>
</tr>
<tr>
<td>Right hippocampal activation</td>
<td>0.326 (0.730; −1.106 – 2.059)</td>
<td>0.567 (0.799; 0.798 – 2.149)</td>
<td>1.714</td>
<td>0.195</td>
</tr>
<tr>
<td><strong>Functional connectivity</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left hippocampus with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right posterior cingulatea</td>
<td>0.094 (0.084 – 0.124)</td>
<td>0.083 (0.067 – 0.108)</td>
<td>10.712</td>
<td>0.002</td>
</tr>
<tr>
<td>Left thalamusa</td>
<td>0.093 (0.080 – 0.116)</td>
<td>0.080 (0.065 – 0.108)</td>
<td>4.984</td>
<td>0.028</td>
</tr>
<tr>
<td>Left inferior temporal lobea</td>
<td>0.092 (0.076 – 0.114)</td>
<td>0.076 (0.067 – 0.096)</td>
<td>4.802</td>
<td>0.031</td>
</tr>
<tr>
<td>Right hippocampus with:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right caudate nucleusa</td>
<td>0.081 (0.068 – 0.103)</td>
<td>0.067 (0.061 – 0.087)</td>
<td>5.415</td>
<td>0.023</td>
</tr>
<tr>
<td>Left Heschl’s gyrusa</td>
<td>0.087 (0.071 – 0.101)</td>
<td>0.072 (0.063 – 0.094)</td>
<td>4.981</td>
<td>0.029</td>
</tr>
<tr>
<td>Left anterior cingulatea</td>
<td>0.087 (0.072 – 0.104)</td>
<td>0.076 (0.062 – 0.093)</td>
<td>4.685</td>
<td>0.034</td>
</tr>
<tr>
<td>Left amygdalaa</td>
<td>0.112 (0.087 – 0.152)</td>
<td>0.089 (0.080 – 0.106)</td>
<td>4.363</td>
<td>0.040</td>
</tr>
<tr>
<td>Left nucleus accumbensa</td>
<td>0.080 (0.069 – 0.100)</td>
<td>0.075 (0.065 – 0.086)</td>
<td>4.040</td>
<td>0.048</td>
</tr>
</tbody>
</table>

aNon-normally distributed variables for which median and interquartile range are presented.
HCs: healthy controls; mL: milliliters; MS: multiple sclerosis.

Connectivity were significant in the regression model, indicating that both measures have relatively independent relevance for memory performance. Because no significant correlation was detected between hippocampal activation and hippocampal connectivity, the changes in these measures could have appeared separately. Additionally, as both measures related to cognition in opposite directions, they perhaps do not reflect the same process in MS.

It does not come as a surprise that local decreases in hippocampal activation coincide with worse memory performance. From studies in healthy ageing, it is known that cognitive decline in the elderly is accompanied by a significant loss of hippocampal activation.28 Additionally, several studies showed increased hippocampal activation in the preclinical stages of cognitive impairment, which is suggested to be a compensatory mechanism to preserve cognitive function for as long as possible.9,29

Besides local changes in hippocampal activation, increased functional connectivity between the left hippocampus and right posterior cingulate reveals global disturbances within a wider memory network. The hippocampus and cingulate play an important role in cognition and are both part of the default mode network.30 Pathological changes in these two structures, and in the default mode network as a whole, are frequently described in Alzheimer’s disease.31 Increased connectivity between the hippocampus and the posterior cingulate within the default mode network was even shown to be a marker of Alzheimer’s disease.32 We suggest that the increased functional connectivity seen in patients with MS in this study was a maladaptive response of the memory network to pathology, possibly driven by disinhibition of the entire default mode network. Increased functional connectivity was previously hypothesized to be maladaptive by other studies, hinting towards the unfavorable consequences of increased functional connectivity, with regard to cognitive functioning in MS (e.g. worse PASAT performance).33 Most research found that an increase in brain activation is beneficial (i.e. related to better functioning),9 although other studies claimed the opposite.34 Our data suggests that there is a distinction between local beneficial increases in activation, versus more diffuse increases in connectivity. In order to elucidate these changes in the brain in more detail, longitudinal studies are now essential, to investigate how and when local changes in activation lead to global network changes, or whether these changes occur in parallel. This might lead to an altered concept of functional reorganization and plasticity in MS.35
In contrast to previous studies, it is of high interest that the structural hippocampal measures were not retained as independent predictors of memory function in MS, although a significant difference was detected between patients and controls in hippocampal volume and presence of hippocampal lesions; however, both functional hippocampal measures (activation and connectivity) were designated as more significant predictors of memory performance than the structural hippocampal measures. Our results do not suggest that structural abnormalities are not related to memory performance in MS, but rather that functional measures of the hippocampus explain more of the variance in memory performance.

A few limitations apply to this work. The current patient group was heterogeneous in terms of disease characteristics (disability scores, disease course and disease duration), which makes it difficult to investigate the activation-connectivity hypothesis for different MS phenotypes. For future studies, inclusion of a more homogenous group of MS patients, or inclusion of a larger number of patients, is therefore recommended. Secondly, hippocampal lesion number (but not volume) was included in the regression analysis. Unfortunately, the double inversion recovery sequence currently does not yet allow for reliable volumetric measurements, due to the indistinctness of lesion borders compared to normal-appearing gray matter. Finally, we did not use a standardized neuropsychological test battery, because we wanted to specifically detect and unravel memory deficits in MS.

In conclusion, future studies should investigate functional connectivity during specific tasks and compare these to the resting-state, and try to confirm the present findings in an independent MS sample. A next study could include tractography, to provide additional information on more subtle changes in structural hippocampal connectivity and expand the arsenal of structural measures. Increasing our understanding of the underlying neurobiological correlates of memory impairment in MS will ultimately help to guide memory rehabilitation studies, which are currently unfortunately still lacking.

Conflict of interest
The authors declare that there are no conflicts of interest.

Funding
This work was supported by the Dutch MS Research Foundation (grant numbers 02–358b, 05–358c, 08–648 and 08–650).

References

Note
Authors Hulst and Schoonheim contributed equally to the manuscript.


