Regional hippocampal atrophy in multiple sclerosis

N. L. Sicotte,1,2 K. C. Kern,1 B. S. Giesser,1 A. Arshanapalli,1 A. Schultz,1 M. Montag,1 H. Wang3 and S. Y. Bookheimer4

1Department of Neurology, 2Division of Brain Mapping, 3Department of Biomathematics and 4UCLA Neuropsychiatric Institute, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Correspondence to: Nancy L. Sicotte, MD, 710 Westwood Blvd. Rm 4-238, Los Angeles, CA 90095, USA
E-mail: nsicotte@ucla.edu

Gray matter brain structures, including deep nuclei and the cerebral cortex, are affected significantly and early in the course of multiple sclerosis and these changes may not be directly related to demyelinating white matter lesions. The hippocampus is an archicortical structure that is critical for memory functions and is especially sensitive to multiple insults including inflammation. We used high-resolution MR imaging at 3.0 T to measure hippocampal volumes in relapsing remitting MS (RRMS) and secondary progressive MS (SPMS) patients and controls. We found that both groups of MS patients had hippocampal atrophy and that this volume loss was in excess of global brain atrophy. Subregional analysis revealed selective volume loss in the cornu ammonis (CA) 1 region of the hippocampus in RRMS with further worsening of CA1 loss and extension into other CA regions in SPMS. Hippocampal atrophy was not correlated with T2-lesion volumes, and right and left hippocampi were affected equally. Volume loss in the hippocampus and subregions was correlated with worsening performance on word-list learning, a task requiring memory encoding, but not with performance on the Paced Auditory Serial Addition Task (PASAT), a test of information processing speed. Our findings provide evidence for selective and progressive hippocampal atrophy in MS localized initially to the CA1 subregion that is associated with deficits in memory encoding and retrieval. The underlying histopathological substrate for this selective, symmetric and disproportionate regional hippocampal vulnerability remains speculative at this time. Further understanding of this process could provide targets for therapeutic interventions including neuroprotective treatments.

Keywords: multiple sclerosis; hippocampus; volumetric imaging; verbal memory

Abbreviations: MS = multiple sclerosis; CA = cornu ammonis; PASAT = paced auditory serial addition task; FLAIR = fluid attenuated inversion recovery

Received October 18, 2007. Revised January 10, 2008. Accepted February 11, 2008

Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disorder affecting the CNS (Noseworthy et al., 2000). The disease causes widespread damage well beyond the traditionally defined white matter lesion areas, leading to diffuse changes in the brain and spinal cord. Compared to age-matched controls, MS patients have decreased brain volumes, ventricular enlargement and spinal cord atrophy (Miller et al., 2002). Gray matter regions of the brain are involved early and significantly in MS as shown by cortical thinning measured on MRI (Sailer et al., 2003) and large areas of cortical demyelination on histopathological section (Peterson et al., 2001). Deep nuclei are atrophied and are abnormal when assessed with non-conventional MR techniques (Wylezinska et al., 2003).

Although other gray matter regions have been studied, relatively little is known of the extent of hippocampal involvement in MS. Demyelinating lesions have been seen in pathological specimens (Vercellino et al., 2005; Geurts et al., 2007) and areas of signal abnormality have been detected within the hippocampus using double inversion recovery MR imaging (Roosendaal et al., 2006). In addition, increased levels of inositol in the hippocampus have been detected using MR spectroscopy, possibly reflecting gliosis (Geurts et al., 2006).

The hippocampus is a complex archicortical structure situated within the medial temporal lobe composed of several subregions with differing histology, functional specificity and susceptibility to disease that plays a critical role in episodic memory formation and retrieval.
Impaired cognitive function is present in 25–60% of MS patients (Rao et al., 1991), can occur in the setting of relatively mild physical disability, and is detectable even before a definitive diagnosis of MS is made (Achiron and Barak, 2003). Although information-processing speed and working memory are the most frequently detected cognitive deficits in MS (Rao et al., 1991) verbal and spatial learning are also affected, possibly reflecting hippocampal dysfunction (Thornton et al., 2002). There are modest correlations of processing speed performance with T2-lesion burden and global brain atrophy measures, but there is a lack of specificity in lesion location with patterns of cognitive dysfunction, suggesting that factors other than white matter lesions or global atrophy contribute to cognitive impairment in MS (Rao et al., 1989; Benedict et al., 2006).

Using high-resolution imaging at 3 T we assessed specific subregions of the hippocampus using a previously described approach (Zeineh et al., 2001) to determine the extent and pattern of hippocampal atrophy in MS patients with relapsing remitting MS (RRMS), secondary progressive MS (SPMS) and a group of healthy controls. At the same visit, we measured performance on screening tests of working and verbal memory to determine if cognitive performance was correlated with hippocampal volumes in MS patients.

**Methods**

**Subjects**

Subjects were recruited from the UCLA Multiple Sclerosis clinic and from the community using the resources of the local chapter of the National MS Society and by placing flyers and contacting local neurologists. The research protocol was approved by the UCLA Human Subjects Protection Committee and informed consent was obtained. Patients studied had clinically definite MS by both Poser and McDonald criteria (Poser et al., 1983; McDonald et al., 2001). Two groups were studied, RRMS with disease duration of <5 years since diagnosis, and SPMS, defined as having worsening neurological functioning over the course of 1 year evidenced by an increase in EDSS of 0.5 or more that was not the result of a relapse (Lublin and Reingold, 1996; Panitch et al., 2004). Patients who had a relapse and/or received steroids within the previous 3 months were excluded. Subjects with a history of drug or alcohol abuse within the last 3 years were excluded. Control subjects were free of any neurological or medical conditions, were on no medications, and had normal neurological examinations.

**Cognitive tasks**

All subjects performed the 3-s version of the Paced Serial Auditory Addition Task (PASAT), a task in which a series of single digit numbers are presented verbally, the two most recent digits are summed, and the answer is reported verbally. All subjects performed the PASAT weekly for three sessions prior to baseline testing to eliminate practice effects (Cohen et al., 2001). On the subsequent baseline visit, the EDSS was calculated and the PASAT was performed, this baseline PASAT result was used for all subsequent analyses. Previous studies have shown that the hippocampus is engaged during verbal learning tasks such as word-pair learning and that performance is affected by hippocampal pathology (Fernandez et al., 1998; Starkman et al., 2003). Therefore, to assess hippocampal function, we also tested the subjects during the same visit with a word-pair learning task that consisted of seven pairs of unrelated words (example—boots: moon). Each set of seven word pairs was presented for a total of six trials. During each learning trial, the entire list of words was read aloud, followed by a prompt using the first word of each pair (example—boots: ?). The subject was required to recall the second word of each of the word pairs. The number of word pairs correctly recalled for each trial and the total number of trials needed for successful learning was recorded. Subjects who were unable to successfully learn all the seven word pairs in six trials were given a score of 10.

**MR imaging**

Imaging was performed on a Siemens 3.0 T Allegra unit. A high-resolution coronal EPI structural sequence was obtained for the structural segmentation procedures (TR 5200/TE 64/3 mm thick, 0 mm spacing/FOV 20 cm/2 NEX/16 slices) with in-plane resolution of 400 μm × 400 μm (see Fig. 1 for scan localization). This T2-weighted scan has high gray-white matter contrast and low-susceptibility artifact and has been optimized for the manual segmentation procedure described later. A T1-weighted three-dimensional volume scan (MPRAGE, TR 1900/TE 4.38/1 mm3/FOV 25.6/1 NEX/160 axial slices) was also acquired to aid in sulcal visualization during segmentation procedures and to determine brain volume measures. In addition, a fluid attenuated inversion recovery (FLAIR) scan was acquired to quantify white matter lesion volumes (TR 10380/TE 88/TI 2500/3 mm thick, 0 mm spacing/FOV 25.6/50 slices).

**Hippocampal segmentation procedures**

Digital image data was transferred to a Macintosh workstation for analysis. The high-resolution T2-weighted coronal scan was displayed and landmarks identified beginning in the most anterior slice. Specific rules for segmentation were based on a histological atlas (Amaral and Insausti, 1990) and have been implemented to reduce inter and intra-rater variability (Zeineh et al., 2001). Absolute volume measurements (in mm3) were determined for each of the four regions within the medial temporal lobe including:

1. Cornu Ammonis 1 (CA1).
2. CA2, CA3 and the Dentate Gyrus considered as a single region (CA23DG).
3. Subiculum (SUB).
4. Entorhinal Cortex (ERC).

Figure 2 shows an example of the hippocampal segmentation technique. Measures from all four regions were combined to yield a total hippocampal volume score for each hemisphere. The totals of the left and right hippocampus were added to yield a total hippocampal volume. Researchers processing image data were blind to group assignment throughout the study. A single investigator with experience in this technique performed all of the segmentations. The intra-rater coefficient of variation was 0.69% for total hippocampal volumes and 0.99% for CA1 regional volumes. T2-lesion volumes were quantified on the FLAIR scan by...
a single experienced researcher using a semi-automated threshold-based algorithm (Shattuck and Leahy, 2002).

Brain volume measurements

Brain volumes were determined for each subject from the T1-volumetric MRI using a fully automated technique, Structural Image Evaluation, using Normalization, of Atrophy for cross-sectional studies (SIENAX) (Smith et al., 2002).

The volume-correction factor was used for the normalization procedure described later. The brain percentage, which is calculated by dividing brain tissue volume by the sum of brain tissue volume and CSF volume was also determined using SIENAX.

Normalization procedures

To control for variations in head size that influence hippocampal size, absolute hippocampal volumes were multiplied by the volume-correction factor determined by SIENAX, yielding corrected hippocampal volumes. All volumetric data and correlations with clinical measures were performed on these hippocampal volumes corrected for head size.

Statistical analysis

Descriptive statistics, such as mean standard deviation, frequency and percentage were used to summarize all outcome measurements. Comparisons between groups (controls versus RRMS versus SPMS) were performed using the general linear model for all brain volume measurements, performance on the PASAT and word-pair learning scores with age and gender effects adjusted. Differences in disease duration, T2 lesions and EDSS between RRMS and SPMS were assessed using the Wilcoxon ranked sums test. Spearman’s rank correlation coefficients were determined to assess the relationship of clinical variables to hippocampal volumes corrected for head size only. All tests were two-sided with significance level 0.05, without correction for multiple comparisons. Statistical software SAS was used for data analysis.

Results

A total of 23 patients with RRMS, 11 with SPMS and 18 healthy controls were enrolled (see Table 1 for clinical
There was no gender difference between the three groups (Chi-square test exact \( P = 0.39 \)). The SPMS patients were significantly older than both the controls (\( P = 0.006 \)), and the RRMS patients (\( P = 0.047 \)), but there was no significant age difference between the RRMS patients and the controls. All subsequent analyses were adjusted for age and gender effects.

Not surprisingly, the SPMS patients had significantly longer disease duration, higher EDSS scores and larger \( T_2 \) lesion volumes than the RRMS patients. PASAT performance was significantly worse in the SPMS group (\( P = 0.005 \) general linear model with age and gender adjusted) but not in the RRMS patients (\( P = 0.22 \)) compared to the healthy controls. Performance on word-list learning, a task that is known to engage the hippocampus, showed a progressive worsening with disease severity in MS as demonstrated by the need for significantly more trials for successful word-list learning in both patient groups compared to controls (Fig. 3). Of the 18 control subjects, only one was unable to learn the seven word pairs within the six-trial learning period (5.5%). In the patient groups, 5 of the 23 RRMS patients (21.7%) and 6 of the 11 SPMS patients (54.5%) failed to successfully learn the word pairs in the allotted number of trials.

Mean brain percentage as determined by SIENAX was decreased in the RRMS (\( P = 0.024 \)) and SPMS (\( P < 0.001 \)) patients compared to the controls reflecting progressive brain atrophy with increased disease duration and severity.

### Table 1 Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Controls (( N = 18 ))</th>
<th>RRMS (( N = 23 ))</th>
<th>SPMS (( N = 11 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age (range)</strong></td>
<td>38 (25–57)</td>
<td>42 (23–54)</td>
<td>49 (34–61)*</td>
</tr>
<tr>
<td><strong>Females/males</strong></td>
<td>13/5</td>
<td>19/4</td>
<td>10/1</td>
</tr>
<tr>
<td><strong>Mean disease duration in years (range)</strong></td>
<td>N/A</td>
<td>3.5 (2–5)</td>
<td>13 (6–21)*</td>
</tr>
<tr>
<td><strong>Mean EDSS (range)</strong></td>
<td>N/A</td>
<td>1.8 (1–3.0)</td>
<td>5.2 (2.5–7)*</td>
</tr>
<tr>
<td><strong>( T_2 ) Lesions volumes in cc(^3)</strong></td>
<td>N/A</td>
<td>10.8 (0.5–45.7)</td>
<td>36.8 (11.9–83.3)*</td>
</tr>
<tr>
<td><strong>Mean score (SD) PASAT (^3)</strong></td>
<td>58.1 (0.6)</td>
<td>53.6 (1.5)</td>
<td>44.9 (4.8)</td>
</tr>
<tr>
<td><strong>Mean number of trials (SD) Needed for word list learning</strong></td>
<td>4.1 (0.45)</td>
<td>5.7 (0.53)</td>
<td>7.7 (0.8)</td>
</tr>
<tr>
<td><strong>Mean brain percentage (range)</strong></td>
<td>0.88 (0.85–0.90)</td>
<td>0.86 (0.83–0.90)</td>
<td>0.82 (0.76–0.87)</td>
</tr>
<tr>
<td><strong>Mean total hippocampal volume in mm(^3)</strong> (range)—corrected for head size</td>
<td>7674 (6543–9219)</td>
<td>6959 (4900–9144)</td>
<td>5817 (4972–6489)</td>
</tr>
</tbody>
</table>

*SPMS versus RRMS, \( P = 0.046 \); SPMS versus Controls, \( P = 0.006 \); RRMS versus Controls = NS (ANOVA).

SPMS versus RRMS, \( P < 0.001 \) (Wilcoxon Rank Sum test).

---

**Fig. 3** MS patients show impairments in PASAT and word-list performance. (A) Performance Results of The Paced Auditory Serial Addition Test (PASAT) administered weekly for 4 weeks are shown for all three groups. RRMS patients show the expected learning effect similar to the control group. SPMS performance was significantly worse at each time point. Means and standard errors shown. Total possible score = 60. Baseline performance: RRMS versus controls \( P = 0.21 \), SPMS versus controls \( P = 0.005 \), SPMS versus RRMS \( P = 0.033 \). Adjusted for age and gender. \(^*\)\(^P < 0.05\) SPMS versus controls at each time point. Wilcoxon rank sum test. (B) Unrelated word-list verbal learning. Mean number of trials needed for successful word-pair learning in all three groups tested. Six trials were allowed for learning. A score of 10 was given for subjects who were still unsuccessful after six trials. Means and standard errors are shown for each group. \(^*\)\(^P = 0.018\) RRMS versus controls. \(^**\)\(^P = 0.002\) SPMS versus controls. Wilcoxon rank sum test.

---

**Table 1 Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Controls (( N = 18 ))</th>
<th>RRMS (( N = 23 ))</th>
<th>SPMS (( N = 11 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age (range)</strong></td>
<td>38 (25–57)</td>
<td>42 (23–54)</td>
<td>49 (34–61)*</td>
</tr>
<tr>
<td><strong>Females/males</strong></td>
<td>13/5</td>
<td>19/4</td>
<td>10/1</td>
</tr>
<tr>
<td><strong>Mean disease duration in years (range)</strong></td>
<td>N/A</td>
<td>3.5 (2–5)</td>
<td>13 (6–21)*</td>
</tr>
<tr>
<td><strong>Mean EDSS (range)</strong></td>
<td>N/A</td>
<td>1.8 (1–3.0)</td>
<td>5.2 (2.5–7)*</td>
</tr>
<tr>
<td><strong>( T_2 ) Lesions volumes in cc(^3)</strong></td>
<td>N/A</td>
<td>10.8 (0.5–45.7)</td>
<td>36.8 (11.9–83.3)*</td>
</tr>
<tr>
<td><strong>Mean score (SD) PASAT (^3)</strong></td>
<td>58.1 (0.6)</td>
<td>53.6 (1.5)</td>
<td>44.9 (4.8)</td>
</tr>
<tr>
<td><strong>Mean number of trials (SD) Needed for word list learning</strong></td>
<td>4.1 (0.45)</td>
<td>5.7 (0.53)</td>
<td>7.7 (0.8)</td>
</tr>
<tr>
<td><strong>Mean brain percentage (range)</strong></td>
<td>0.88 (0.85–0.90)</td>
<td>0.86 (0.83–0.90)</td>
<td>0.82 (0.76–0.87)</td>
</tr>
<tr>
<td><strong>Mean total hippocampal volume in mm(^3)</strong> (range)—corrected for head size</td>
<td>7674 (6543–9219)</td>
<td>6959 (4900–9144)</td>
<td>5817 (4972–6489)</td>
</tr>
</tbody>
</table>

*SPMS versus RRMS, \( P = 0.046 \); SPMS versus Controls, \( P = 0.006 \); RRMS versus Controls = NS (ANOVA).

SPMS versus RRMS, \( P < 0.001 \) (Wilcoxon Rank Sum test).
After correction for head-size differences, total hippocampal volumes were smaller in both the RRMS ($P = 0.004$) and SPMS group ($P < 0.001$) compared to controls. Examination of individual hippocampal subregions revealed that in the RRMS patients, hippocampal volume loss was localized to the CA1 region. On average, this represented a 12% decrease in CA1 volume in RRMS compared to control values. In the SPMS group, a further decline in CA1 volume was seen, representing an average loss of 25% compared to controls. In addition, in the SPMS group, volume was decreased significantly in the CA23DG region (13% loss). Subiculum and entorhinal cortical volumes were relatively preserved in both groups of patients consistent with a selective vulnerability in the areas that include the CA fields. Combined values are shown here but results were the same for both right and left hippocampi. CA1 = Cornu ammonis 1; CA23 DG = Cornu ammonis 2&3 and dentate gyrus considered as a single subregion; Sub = Subiculum; ERC = Entorhinal cortex.

$P < 0.0001$ RRMS versus controls. $**P < 0.0001$ SPMS versus controls. $P < 0.004$ SPMS versus controls. All analyses corrected for age/gender.

Fig. 4 Progressive regional hippocampal volume loss in MS. Mean volumes for total hippocampal volume and in each of four hippocampal subregions after correction for head size only are shown. (A) Total hippocampal volume is decreased in both RRMS and SPMS compared to controls. $*P = 0.0063$ RRMS versus controls. $**P < 0.0001$ SPMS versus controls. (B) Examining specific subregions shows that RRMS patients have a significant and selective volume loss in the CA1 region compared to controls (average decrease of 12%). SPMS patients show further worsening of CA1 atrophy (average decrease of 25%) with extension into the other hippocampal regions. Results are unchanged after correction for global brain atrophy consistent with a selective volume loss in the hippocampal regional loss that is in excess of global brain atrophy. Means and standard deviations are shown for combined volumes of both right and left hippocampi. CA1 = Cornu ammonis 1; CA23 DG = Cornu ammonis 2&3 and dentate gyrus considered as a single subregion; Sub = Subiculum; ERC = Entorhinal cortex.

There was no correlation between EDSS in either the RRMS or the SPMS patients. Total T2-lesion volumes were not significantly correlated with either total or subregional hippocampal volumes in the RRMS patients. However, when limiting the analysis to the SPMS patients alone, there was a significant correlation of T2 volumes with CA1 atrophy ($r = -0.69, P = 0.018$).

No areas of high signal were detected in the hippocampal gray matter, but visualization of cortical lesions may require other techniques such as double inversion recovery scanning (Geurts et al., 2005; Wattjes et al., 2007). In 6 of the 23 RRMS patients and 4 of the 11 SPMS patients, there was evidence of white matter demyelination adjacent to the hippocampus, in the medial temporal lobe. Eliminating the subjects who had demyelinating lesions in the medial temporal white matter did not change the findings of CA1 or total hippocampal volume loss.

Ten of the 23 RRMS patients had exposure to high-dose steroids, but there was no correlation of steroid exposure with CA1 volumes. Eliminating the 10 subjects with steroid exposure did not change the results (data not shown). The majority of the SPMS patients (8 of the 11) had received at least one course of steroids (median number of courses = 1.0, range 0–6).

There was no correlation between PASAT performance and either total or subregional hippocampal volumes in the patient groups. However, there was a significant, inverse relationship between the number of trials needed for word-list learning and total hippocampal volume, as well as with CA1 and subiculum subregional volumes in the MS patients.
and this relationship was stronger for the left hippocampus, as seen in Table 2 (see also Fig. 5).

**Discussion**

Our data show early, selective volume loss in the CA1 region of the hippocampus in RRMS that is disproportionate to global brain atrophy. This regional CA1 hippocampal atrophy worsens in SPMS with extension into other CA regions. Both regional and total hippocampal volume loss is associated with worsening performance on screening tests of verbal learning but not processing speed. Word-list learning is most closely associated with left total and subregional hippocampal volumes, consistent with the verbal nature of the task. Interestingly, the subiculum, which is the major target of CA1 projections (Lavenex and Amaral, 2000), is also correlated with word-list-learning performance. Determining the specificity of these findings will require more complete testing of multiple cognitive domains including tasks of spatial memory, in association with right hippocampal volumes.

The volume loss detected in this study could reflect a variety of neuropathological processes including demyelination, decreased dendritic density and/or neuronal loss. Notably, neurons within the CA1 region are highly vulnerable to a variety of insults including ischaemia and glutamate-mediated excitotoxicity (Wang *et al.*, 2005), which has been implicated in MS-related CNS damage (Vallejo-Illarramendi *et al.*, 2006). Exposure to high-dose corticosteroids, used frequently to treat acute relapses, is another potentially important variable that could impact hippocampal volume. Corticosteroids have pronounced effects on CA1 neurons (Lu *et al.*, 2003; Crochemore *et al.*, 2005), and MS patients treated with high-dose steroids have transient deficits in declarative memory (Brunner *et al.*, 2005; Roozendaal and de Quervain, 2005; Uttner *et al.*, 2005). In our group of 23 early RRMS patients, steroid exposure was relatively low, with more than half of the patients having never received any steroids at all.

High levels of endogenous cortisol have also been associated with deleterious effects on hippocampal neurons (MacPherson *et al.*, 2005) and have been found in MS patients (Michelson *et al.*, 1994). However, in most reports, high levels of endogenous glucocorticoids have effects

<table>
<thead>
<tr>
<th>Table 2 Correlation between total and subregional hippocampal volumes and cognitive test performance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hippocampal region</strong></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Left and right</td>
</tr>
<tr>
<td>CA1</td>
</tr>
<tr>
<td>CA23DG</td>
</tr>
<tr>
<td>SUB</td>
</tr>
<tr>
<td>ERC</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Right</td>
</tr>
<tr>
<td>CA1</td>
</tr>
<tr>
<td>CA23DG</td>
</tr>
<tr>
<td>SUB</td>
</tr>
<tr>
<td>ERC</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>Left</td>
</tr>
<tr>
<td>CA1</td>
</tr>
<tr>
<td>CA23DG</td>
</tr>
<tr>
<td>SUB</td>
</tr>
<tr>
<td>ERC</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

Results shown are for RRMS and SPMS patients combined. NS = not significant.

Fig. 5 Atrophy of total and CA1 hippocampal volumes is associated with worsening word-list learning. Boxplots of word-list-learning performance and both CA1 (top panel) and total (bottom panel) hippocampal volume measurements arranged from the lowest (1st quartile) to the highest (4th quartile) volumes. Patients with lower hippocampal volumes required more learning trials for the unrelated word-pair task. Patients were divided into four groups according to corrected hippocampal volumes using the 25, 50 and 75th percentiles as the cutoff points. The length of box represents the interquartile range (between 25th and 75th percentiles), the centre dot is the mean and the horizontal line is the median. Vertical lines are minimum and maximum values.
localized to the CA3 region of the hippocampus (Conrad, 2006; Tata et al., 2006). Whether and to what extent the CA2 and CA3 regions might be affected in these patients cannot be directly addressed by these data, due to the lack of sufficient resolution with MR scanning to assess these regions separately from the larger dentate gyrus. The results reported combine CA2, CA3 and the dentate gyrus into a single region, therefore selective volume loss within one or both of the CA regions in the RRMS patients could be masked by relative preservation of the dentate gyrus. Cortisol levels were not measured in this group of patients, but could be informative for future studies. Overall, our findings of hippocampal atrophy in patients with MS are not explained by exogenous steroid exposure, and the pattern detected is not consistent with an effect due to high levels of endogenous steroids.

Although no areas of T2-or FLAIR abnormality were detectable within the hippocampi of the patients studied, it is possible that occult demyelinating lesions were present within the hippocampus and accounted for these findings. A recent neuropathological study has found evidence of demyelination including intracortical lesions within the hippocampus in 15 of 19 chronic MS patients (Geurts et al., 2007). Interestingly, there was some regional selectivity with a relative sparing of CA2 and the dentate gyrus even in these progressed patients. The presence of demyelination in the hippocampus in MS patients has also been correlated with significant atrophy and associated neuronal loss (Dukes et al., 2007). In contrast, neocortical demyelination leads to atrophy that is due more to decreased synaptic density (up to 47%) than neuronal loss (10% decrease in neuronal density) (Wegner et al., 2006). Interestingly, a loss of hippocampal synaptic density has been associated with worsening cognitive function in Alzheimer’s disease (Terry et al., 1991; Sze et al., 1997). Studies of the thalamus, another deep gray matter structure affected by MS, have shown that atrophy is associated with significant neuronal loss (Cifelli et al., 2002). These findings suggest that a variety of pathological processes can lead to atrophy of cortical and deep gray matter structures in MS.

We found no correlation of hippocampal volume loss with total T2 white matter lesion volumes in the RRMS patients, however, the extent of cortical demyelination and white matter lesions may not be closely linked (Roosendaal et al., 2006). It is possible that the hippocampal volume loss detected in our subjects is a result of cortical demyelination, but the finding that atrophy affected both hippocampi selectively and symmetrically and was detectable in the early stages of RRMS in which extensive cortical demyelination has not been reported, raises the possibility that other disease processes may be involved, at least in the earlier stages of disease.

These findings are further evidence that there may be tissue-specific vulnerabilities to the disease process in MS that are not directly related to white matter demyelinating lesions. Whether and to what extent the hippocampal volume changes seen represent demyelination, synaptic loss and/or irreversible neuronal loss will require further study using pathological specimens in early RRMS patients and longitudinal in vivo MRI studies.

In conclusion, we have found evidence of selective atrophy within the hippocampus in MS that is associated with impaired performance on a cognitive test of verbal memory. These findings could implicate distinct disease mechanisms leading to gray matter loss in MS and are consistent with emerging evidence of an early neurodegenerative process affecting the gray matter in MS (Pirko et al., 2007). A better understanding of hippocampal damage in MS could lead to specific neuroprotective treatment strategies in these patients.

**Acknowledgements**

This work was supported by the National Multiple Sclerosis Society (JF2107, RG3914), the Skirball Foundation and generous donations from Claire and William Vaughn.

**References**


Geurts JJ, Reuling IE, Vrenken H, Uitdehaag BM, Polman CH, Castelijns JA, et al. MR spectroscopic evidence for thalamic and