Entorhinal cortex atrophy in epilepsy patients exhibiting normal hippocampal volumes

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Article abstract—Objective: To determine whether MRI volumetric measurement of the entorhinal cortex could detect structural damage and lateralize the seizure focus in patients with temporal lobe epilepsy in whom no measurable hippocampal abnormalities were found. Background: A reduction in the volume of the entorhinal cortex ipsilateral to the seizure focus in patients with intractable temporal lobe epilepsy and hippocampal atrophy was recently shown. Methods: MRI volumetric analysis of the entorhinal cortex was performed using a T1-weighted three-dimensional gradient echo sequence in 24 control subjects and 22 patients with temporal lobe epilepsy and normal hippocampal volumes. Thirteen patients underwent surgery, with a mean postoperative follow-up of 36 months. Results: Group analysis (multivariate analysis of variance) showed a reduction in the volume of the entorhinal cortex ipsilateral to the seizure focus in patients with left ($p < 0.0001$) and right temporal lobe epilepsy ($p < 0.0001$). Lateralization of the seizure focus could be done in 14 of 22 patients (64%) based on entorhinal cortex volumetry. Conclusion: Entorhinal cortex atrophy ipsilateral to the seizure focus supports the presence of structural damage in the mesial temporal lobe in patients with temporal lobe epilepsy and normal hippocampal volumes and emphasizes the participation of the entorhinal cortex in the pathogenesis of this disorder.

Hippocampal sclerosis is the most common pathology underlying pharmacologically intractable temporal lobe epilepsy (TLE). For the past 10 years, advances in neuroimaging have had a major impact on in vivo detection of hippocampal sclerosis. A common MRI finding in patients with TLE is hippocampal atrophy on T1-weighted images. The sensitivity of MRI visual analysis can be increased by the use of quantitative MRI techniques. Because it is relatively easy to diagnose hippocampal sclerosis and quantitate hippocampal atrophy preoperatively, MRI studies in TLE have put the emphasis on the hippocampus, and little attention has been paid to changes in the remaining components of medial temporal lobe, such as the entorhinal cortex (EC).

Hippocampal atrophy is found in 85% of patients with TLE, and the epileptic focus is lateralized in about 80% of patients. Hippocampal volumes are normal in about 15% of patients with intractable TLE, even though postoperative specimens from the majority of these individuals demonstrate histopathologic evidence of hippocampal sclerosis.

We recently showed a reduction in the volume of the EC ipsilateral to the seizure focus in patients with intractable TLE and hippocampal atrophy. We hypothesized that MRI volumetric measurement of the EC could detect structural damage in patients with TLE in whom no measurable hippocampal abnormalities are found. Furthermore, we were interested in determining the usefulness of measuring EC volume in the lateralization of the epileptic focus in this group of patients.

Methods. Subjects. We selected 22 patients with medically intractable TLE (mean age, 34 years; range, 21 to 50) and normal hippocampal and amygdalar volumes on volumetric MRI. These 22 patients were compared with 24 subjects without neurologic symptoms recruited among the employees of our Brain Imaging Center (mean age, 30 years; range, 20 to 51). Lateralization of seizure focus. Seizure type and site of seizure onset were determined by a comprehensive evaluation that included detailed history taking, neurologic examination, review of medical and EEG records, and neuropsychological evaluation. The seizure focus was determined by predominantly ipsilateral interictal epileptic abnormalities (70% cutoff) by unequivocal unilateral seizure onset recorded during prolonged video EEG monitoring using sphenoidal electrodes. Four patients had depth electrode studies. Based on these criteria, patients with TLE were divided into those with a left-sided ($n = 12$) or a right-sided ($n = 10$) seizure focus. Thirteen patients had surgery: five underwent a selective amygdalohippocampec-
tomy and eight underwent temporal cortical resection with amygdalohippocampectomy. As tissue was removed by subpial aspiration, histopathologic information from the EC was not available. Qualitative histopathologic examination revealed hippocampal sclerosis in nine patients. Specimens for histopathologic assessment of the hippocampus were unsuitable in five patients. Figure 1 illustrates the histopathology of the hippocampus in a patient with normal hippocampal volumes and in a patient with hippocampal atrophy on MRI. Ten patients have been seizure free (Engel class I) since surgery, and three had rare disabling seizures (Engel class II). The mean postoperative follow-up was 36 months (range, 14 months to 6 years).

**MRI scanning.** Images were acquired on a 1.5-T Gyroscan device (Philips Medical System, Eindhoven, the Netherlands), using a T1-weighted, three-dimensional gradient echo sequence (repetition time, 18 ms; echo time, 10 ms; 1 signal average; flip angle 30°; matrix 256 × 256; field of view, 250 mm; slice thickness, 1 mm). Approximately 170 slices with an isotropic voxel size of 1 mm³ were acquired. Images were automatically registered into stereotaxic space to adjust for differences in total brain volume and brain orientation, and to facilitate the identification of boundaries by minimizing variability in slice orientation. This transformation maximized the cross-correlation between each individual’s MR image and a target image consisting of the average of over 300 normal MR images already registered into stereotaxic coordinates. Each image underwent automated correction for intensity nonuniformity (due to radiofrequency inhomogeneity of the MR scanner) and intensity standardization. This correction produces consistent relative gray matter, white matter, and CSF intensities. Volumetric analysis was performed on a Silicon Graphics (Mountain View, CA) workstation. The EC, hippocampus, and amygdala were segmented manually. The EC boundaries were as defined by Bernasconi et al. (figure 2). The hippocampal and the amygdalar boundaries were defined according to Watson et al. The segmentation was done using the mouse-driven software package DISPLAY, developed at the Brain Imaging Center of the Montreal Neurological Institute. This software allows simultaneous viewing of volumes in coronal, sagittal, and horizontal orientations.

**Statistical analysis.** The statistical significance of differences in mean volumes between right and left sides was assessed using the paired Student’s t-test. Group differences for volumes was examined using a multivariate analysis of variance (MANOVA) with one between-subject grouping factor (groups: control subjects, patients with left TLE, patients with right TLE) and two within-subject factors: factor 1, hemisphere (left or right); factor 2, structure (EC, hippocampus, or amygdala). MANOVA was followed by Tukey Honest Significant Difference post hoc comparisons. For each individual, left–right asymmetries in entorhinal, hippocampal, and amygdalar volumes were calculated as follows: (L – R)/L + R/2, where L and R refer to the mean left and right volume of each structure. Group differences in left–right asymmetries were examined using MANOVA with one between-subject grouping factor (group: control subjects, patients with left TLE, patients with right TLE) and one within-subject grouping factor (structure: EC, hippocampus, or amygdala). MANOVA was followed by Bonferroni-corrected planned comparisons. For analysis of individual patients, we considered as abnormal values that were 2 SD below the mean of normal subjects.

**Results.** The mean volumes of the EC, hippocampus, and amygdala in control subjects and patients with TLE

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*Figure 1.* (A) Hematoxylin-eosin stained section of the hippocampus taken from a patient with normal hippocampal volume on MRI shows neuronal loss and gliosis in CA4. Arrowhead shows one surviving neuron. (B) Section stained with glial fibrillary acidic protein in the same patient shows gliosis. (C) Hematoxylin-eosin stained section of the hippocampus at the junction between CA1 and CA2 from a patient with hippocampal atrophy on MRI. Note complete neuronal loss and gliosis in CA1. Arrowheads show surviving neurons in CA2.
Table

<table>
<thead>
<tr>
<th>Area</th>
<th>Control subjects (n = 24)</th>
<th>Patients with right TLE (n = 10)</th>
<th>Patients with left TLE (n = 12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entorhinal cortex, R</td>
<td>1,361 ± 167</td>
<td>1,117 ± 136</td>
<td>1,198 ± 182</td>
</tr>
<tr>
<td>Entorhinal cortex, L</td>
<td>1,305 ± 138</td>
<td>1,222 ± 188</td>
<td>1,039 ± 157</td>
</tr>
<tr>
<td>Hippocampus, R</td>
<td>3,226 ± 370</td>
<td>2,961 ± 291</td>
<td>3,052 ± 310</td>
</tr>
<tr>
<td>Hippocampus, L</td>
<td>3,018 ± 305</td>
<td>2,610 ± 250</td>
<td>2,957 ± 324</td>
</tr>
<tr>
<td>Amygdala, R</td>
<td>2,035 ± 248</td>
<td>2,103 ± 283</td>
<td>2,020 ± 162</td>
</tr>
<tr>
<td>Amygdala, L</td>
<td>1,954 ± 263</td>
<td>1,935 ± 324</td>
<td>2,036 ± 188</td>
</tr>
</tbody>
</table>

TLE = temporal lobe epilepsy.
and hippocampal atrophy are different from patients with TLE without atrophy. None of our patients with normal hippocampal volume had a history of febrile convulsions during childhood. Patients with prolonged febrile convulsions in early childhood have been shown to have a significantly smaller hippocampus ipsilateral to the seizure focus compared with those without such a history.22-24 It may be that the hippocampus is particularly sensitive to febrile convulsions. Consequently, the development of hippocampal sclerosis, and its in vivo correlate hippocampal atrophy, may be more severe in patients who had febrile convulsions as opposed to patients with TLE who did not have prolonged febrile convulsions.

The absence of hippocampal atrophy in our patients does not exclude the possibility that the hippocampus was structurally abnormal. In fact, nine of the 12 patients who underwent surgery had evidence of hippocampal sclerosis based on a qualitative histopathologic analysis. Using quantitative techniques, King et al.7 showed that patients with normal hippocampal volume have less CA1 cell loss and fewer glial cells in CA1 as compared with patients with hippocampal atrophy. Moreover, we previously demonstrated that T2 relaxometry could detect hippocampal abnormalities in patients with TLE and normal hippocampal volumes.25 It may be that in our patients, an undetermined initial injury to the EC contributed to long-lasting changes in excitability and cell loss, which then led to secondary damage to the hippocampus in some patients.

References


