Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region

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Summary
Despite neuropathological and electrophysiological evidence for the involvement of parahippocampal structures in temporal lobe epilepsy (TLE), little attention has been paid to morphometric measurements of these structures in patients with TLE. Using high resolution MRI, we previously showed that the volume of the entorhinal cortex was decreased in patients with TLE. The purpose of this study was: (i) to determine whether changes in the volume of the perirhinal cortex and posterior parahippocampal cortex were detectable by MRI; and (ii) to study the distribution and degree of atrophy in mesial temporal structures including the hippocampal head, body and tail, amygdala, entorhinal cortex, perirhinal cortex and posterior parahippocampal cortex. MRI volumetric analysis was performed using a T_1-weighted three-dimensional gradient echo sequence in 20 healthy subjects and 25 TLE patients with intractable TLE. In patients with either left or right TLE, the hippocampal head, body and tail and the entorhinal and perirhinal cortices ipsilateral to the seizure focus were significantly smaller than in normal controls. The mean volume of the posterior parahippocampal cortex was not different from that of normal controls. Within the hippocampus, the hippocampal head was more atrophic than the hippocampal body and hippocampal tail. Within the parahippocampal region, the entorhinal cortex was more severely affected than the perirhinal cortex. Our MRI results confirm pathological findings of damage in the mesial temporal lobe, involving not only the hippocampus and the amygdala, but also the entorhinal and perirhinal cortices. The pattern of atrophy may be explained by cell loss secondary to a disruption of entorhinal–hippocampal connections as a result of privileged electrical dialogue between these two structures.

Keywords: entorhinal cortex; perirhinal cortex; parahippocampal region; MRI; temporal lobe epilepsy

Abbreviations: EC = entorhinal cortex; HB = hippocampal body; HH = hippocampal head; HT = hippocampal tail; PC = perirhinal cortex; PPC = posterior parahippocampal cortex; TLE = temporal lobe epilepsy

Introduction
The human mesial temporal lobe is composed of the hippocampus, the amygdala and the parahippocampal region. The parahippocampal region comprises several cortical regions grouped together on the basis of their unique laminar organization and connectivity (Scharfman et al., 2000). In its anterior portion, the parahippocampal region includes the entorhinal cortex (EC) and the perirhinal cortex (PC); its posterior portion is composed of the posterior parahippocampal cortex (PPC) (areas TH and TF of von Bonin and Bailey) (Bailey and Von Bonin, 1951).

In earlier studies, most attention was dedicated to the hippocampus, which is believed by many to play a primary role in the genesis of epileptic phenomena. The importance of the parahippocampal region in the genesis of temporal lobe epilepsy (TLE) was underestimated for a long time. However, recent observations in animal models of TLE indicate that the epileptogenic zone is broad, and suggest that the substrate for seizure generation is distributed over several limbic structures (Bertram, 1997), including the entorhinal and perirhinal cortices. There is also indication, from neurosurgical,
neuropathological and electrophysiological studies in humans, that parahippocampal structures are critically involved in TLE (Rutecki et al., 1989; Du et al., 1993; Plate et al., 1993; Spencer and Spencer, 1994).

Detailed descriptions of gross anatomy and cytoarchitectonic borders of parahippocampal structures on histological sections have been produced (Insausti et al., 1993; Spencer and Spencer, 1994), allowing a precise determination of the boundaries of these cortical areas on MRI. Volumetric MRI has been successful in the assessment of hippocampal and amygdalar damage in TLE (Cascino et al., 1991; Cendes et al., 1993; Watson et al., 1997). Quantitative MRI studies of the parahippocampal region in TLE are few (Bernasconi et al., 2000; Jutila et al., 2001). Using high-resolution MRIs, we previously showed a reduction in volume of the EC ipsilateral to the seizure focus in patients with intractable TLE (Bernasconi et al., 1999). Our findings were confirmed in a recent study by Jutila et al. (2001).

The purpose of this study was to complete the study of the parahippocampal region in TLE by examining if volume changes of the PC and PPC are detectable on MRI. This allowed us to study for the first time in vivo the extent of damage within the mesial temporal lobe and to examine the degree of atrophy of its different components. Our preliminary results based on volumetric measurement of the parahippocampal region structures in six TLE patients showed a decrease in the volume of the PC in two of them and no decrease in the volume of the PPC (Bernasconi et al., 2000).

Methods
We studied 25 patients with medically intractable TLE (11 males, mean age ± SD = 37 ± 10 years, range = 18–53). Patients were compared with 20 neurologically normal controls (nine males, mean age = 33 ± 10 years, range = 20–54).

The Ethics Committee of The Montreal Neurological Institute and Hospital (MNI) approved the study and informed consent was obtained from all participants prior to their inclusion.

Lateralization of seizure focus
Seizure type and the site of seizure onset were determined by a comprehensive evaluation including detailed history, neurological examination, review of medical and EEG records, and neuropsychological evaluation. The seizure focus was determined by predominantly ipsilateral interictal epileptic abnormalities (70% cutoff), or unequivocal unilateral seizure onset recorded during prolonged video-EEG monitoring, and confirmed by response to surgical treatment in 23 out of 25. Based on these criteria, TLE patients were divided into those with a left-sided (n = 13) or a right-sided (n = 12) seizure focus. Seventeen patients underwent a selective amygdalo-hippocampectomy, and six underwent anterior temporal lobectomy. When tissue was available (n = 13), qualitative histopathological examination (Meencke and Veith, 1991) revealed hippocampal sclerosis. Histopathologies of the parahippocampal region structures were not available. Twenty-one patients have been seizure free since surgery, and two have rare seizures with a mean postoperative follow-up of 20 months (range = 1–2.5 years).

MRI scanning
MRI volumetric images were acquired on a 1.5 T Gyroscan (Philips Medical System, Eindhoven, The Netherlands), using a T1-fast field echo, repetition time (TR) = 18 ms, echo time (TE) = 10 ms, one acquisition average pulse sequence, flip angle = 30°, matrix size = 256 × 256, field of view (FOV) = 256, thickness = 1 mm. Approximately 170 isotropic images with a voxel size of 1 mm × 1 mm × 1 mm were acquired.

Image processing
Analysis was performed on a Silicon Graphics workstation (Mountain View, CA). Images were registered automatically in a standard, stereotaxic space (Talairach and Tournoux, 1988) to adjust for differences in total brain volume and brain orientation and to facilitate the identification of boundaries by minimizing variability in slice orientation (Collins et al., 1994). This procedure uses only a linear transformation. It has been shown that the automatic stereotaxic transformation is as accurate as the manual procedure, but shows higher stability (Collins et al., 1994). Each image underwent automated correction for intensity non-uniformity due to radiofrequency inhomogeneity of the MRI scanner and intensity standardization (Sled et al., 1998).

Volumetric analysis was performed using an interactive software package DISPLAY developed at the Brain Imaging Center of the Montreal Neurological Institute, Canada. This program allows simultaneous viewing of MRIs in coronal, sagittal and horizontal orientations. The hippocampus, the amygdala (Watson et al., 1992), the EC (Bernasconi et al., 1999), the PC (Insausti et al., 1998b) and the PPC (Insausti et al., 1998a) were segmented manually according to previously described protocols. The hippocampus was divided further into head (HH), body (HB) and tail (HT) according to the anatomical boundaries described by Duvernoy (1988). On coronal sections, one external landmark, the lateral geniculate body, and two internal landmarks, the uncus and the fimbria, mark the junction between the head and the body. As the anterior limit of the HB, we chose the first coronal slice, perpendicular to the AC–PC line, in which the lateral geniculate body and the fimbria became visible. This coincided with the last slice on which the gyrus intralimbicus was visible. The junction between the HB and HT was set to be the slice on which the crus fornix became fully visible (Fig. 1).

Statistical analysis
Group differences for age were assessed using one-way ANOVA (analysis of variance). The gender distribution
was examined by the $\chi^2$ test. The statistical significance of differences in mean volumes between right and left sides was assessed using the paired $t$ test. Each individual’s volume measurements were standardized relative to the value of normal controls using a $Z$-score transformation. For any individual, a $Z$-score of $-1.0$ on any volumetric measure indicates a raw value that is $1$ SD below the mean of normal controls on that measure.

Group differences for volumes were examined using a MANOVA (multivariate analysis of variance) with one between-subjects grouping factor (groups: normal controls, left TLE patients, right TLE patients) and two within-subjects factors (factor 1 = left hemisphere, right hemisphere; factor 2 = structure: HH, HB, HT, amygdala (AM), EC, PC, PPC). The MANOVA was followed by Newman–Keuls post hoc comparisons. For each individual, asymmetries in 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 volume asymmetries were examined using MANOVA with one between-subjects grouping factor (groups = normal controls, left TLE patients, right TLE patients) and one within-subjects factor (structure = HH, HB, HT, AM, EC, PC, PPC).

For analysis of individual patients, we considered as abnormal: (i) values that were $2$ SD below the mean of normal controls; and (ii) left–right asymmetries at least $2$ SD above or below the mean of normal controls.

Fig. 1 Major anatomical boundaries of mesial temporal lobe on coronal MRI. A is the most rostral and H is the most caudal MRI section. Only MRIs displaying critical landmarks are shown. (A) The anterior border of the perirhinal cortex (PC) is located at the level of the limen insulae (LI). (B) The anterior border of the entorhinal cortex (EC) begins on average $2$ mm behind the limen insulae. This coincides with the appearance of the temporal stem (TS). (C) Section through the hippocampal head (HH). (D) The posterior border of the EC is located at the posterior limit of the gyrus intralimbicus (GI) and coincides with the anterior border of the hippocampal body (HB). (E) The posterior border of the PC is situated $2$ mm caudal to the posterior end of the EC. (F) The rostral border of the posterior parahippocampal cortex (PPC) is situated $1$ mm caudal to the posterior end of the PC. (G) The anterior border of the hippocampal tail (HT) coincides with the crus fornix (CF) becoming fully visible. (H) The posterior border of the PPC is situated at the level of the posterior end of the hippocampal tail (HT). AM = amygdala; CS = collateral sulcus; FI = fimbria.
**Results**

The patients with right TLE and left TLE and normal controls did not differ significantly in age [ANOVA; $F(2,42) = 0.82$, $P = 0.5$] or sex distribution [$\chi^2; \chi^2 (1) = 0.034$, $P = 0.8$ for right TLE and $\chi^2; \chi^2 (1) = 0.004$, $P = 0.9$ for left TLE].

### Hippocampal volumes in normal controls

The mean volume of the right HH (2071 ± 298 mm$^3$) was greater than that of the left (1849 ± 254 mm$^3$; $P = 0.001$). The mean volume of the right HB was 913 ± 157 mm$^3$ and that of the left was 917 ± 151 mm$^3$ ($P = 0.9$). The mean volume of the right HT was 324 ± 100 mm$^3$ and that of the left was 311 ± 97 mm$^3$ ($P = 0.2$).

### Amygdalar volumes in normal controls

The mean volume of the right amygdala was 2121 ± 367 mm$^3$ and that of the left amygdala was 2027 ± 319 mm$^3$; $P = 0.1$.

### Parahippocampal region volumes in normal controls

The mean volume of the right EC (1395 ± 148 mm$^3$) was greater than that of the left (1321 ± 123 mm$^3$; $P = 0.02$). The mean volume of the right PC was 2832 ± 437 mm$^3$ and that of the left was 2987 ± 541 mm$^3$ ($P = 0.08$). The mean volume of the left PPC (2384 ± 545 mm$^3$) was greater than that of the right (2226 ± 393 mm$^3$; $P = 0.02$).

### Hippocampal, amygdalar and parahippocampal volumes in TLE patients

Results of group analysis for MRI volumetry of mesial temporal structures are shown in Fig. 2A and B. In patients with left TLE and those with right TLE, HH, HB and HT ipsilateral to the seizure focus were significantly smaller than in normal controls ($P < 0.001$) and there was a significant asymmetry for all three parts of the hippocampus ($P < 0.001$).
In patients with left TLE, the HH was also atrophic contralateral to the seizure focus \( P < 0.001 \). In patients with right TLE, the HB was also atrophic contralateral to the seizure focus \( P = 0.001 \). The amygdala ipsilateral to the seizure focus was significantly smaller than in normal controls \( P = 0.001 \) only in patients with left TLE, and there was also a significant asymmetry \( P < 0.001 \).

Within the parahippocampal region, in both patients with left TLE and those with right TLE, the EC and PC were significantly smaller than in normal controls \( P < 0.001 \) and were significantly asymmetric \( P < 0.001 \). The EC was also atrophic contralateral to the seizure focus in patients with left TLE \( P < 0.001 \). There was no difference between the mean volume of the PPC in TLE patients and that of normal controls. There was a significant asymmetry for the PPC in patients with right TLE (right smaller than left) compared with normal controls \( P = 0.02 \). The asymmetry of the PPC was not significant in patients with left TLE.

**Distribution of mesial temporal atrophy ipsilateral to the epileptic focus**

In both patients with left TLE and those with right TLE, within the hippocampus, the HH was more atrophic than the HB \( P < 0.001 \) and the HB was more atrophic than the HT \( P < 0.001 \).

Within the parahippocampal region, the EC was more atrophic than the PC \( P < 0.001 \) and the PC was more atrophic than the PPC \( P < 0.001 \) in both those with left TLE and those with right TLE. The HH, HB and the EC were the most atrophic structures.

**Individual analysis**

The HH was abnormal in all 25 patients: in 23 out of 25 (92%), the atrophy was unilateral and ipsilateral to the seizure focus, and in two out of 25 (8%), the HH was symmetrically and bilaterally atrophic. The volume of HB was decreased ipsilateral to the seizure focus in 16 out of 25 (64%) patients. The HT was atrophic unilaterally in 14 out of 25 (56%) patients. The volume of the amygdala was decreased ipsilateral to the seizure focus in six out of 25 (24%) patients. The EC was abnormal in 24 out of 25 (96%) patients: in 16 out of 24 (67%), the atrophy was unilateral and ipsilateral to the seizure focus, and in eight out of 24 (33%) the EC was symmetrically and bilaterally atrophic. The PC was atrophic ipsilateral to the seizure focus in 11 out of 25 (44%) patients. The PPC was atrophic ipsilateral to the seizure focus in four out of 25 (16%) patients.

**Discussion**

Because hippocampal sclerosis represents a frequent finding in patients with intractable TLE, and it is relatively easy to evaluate hippocampal pathology *in vivo*, most MRI studies of TLE have assessed the hippocampus, and little attention has been paid to pathological changes in extra-hippocampal limbic structures.

As described in neuropathological studies (Falconer et al., 1964; Meencke and Veith, 1992), our results confirm that damage to the mesial temporal lobe involves not only the hippocampus and the amygdala but also the parahippocampal region structures in patients with intractable TLE. Atrophy is more severe in the anterior portion of the mesial temporal lobe, involving mostly HH, HB and the EC.

We found that HH and EC were bilaterally atrophic only in patients with left TLE. It is not clear whether this represents a tendency for more severe damage in patients with left TLE or is a quirk of the small sample size.

**Distribution of atrophy within the hippocampus**

Previous MRI studies addressing the question of regional distribution of atrophy within the hippocampus in TLE have found dissimilar results, showing either anterior atrophy (Cook et al., 1992; Van Paesschen et al., 1997), atrophy involving mostly the body of the hippocampus (Bronen et al., 1995; Kuzniecky et al., 1996) or diffuse hippocampal volume loss (Quigg et al., 1997; Van Paesschen et al., 1997). The heterogeneity of the results between studies could be explained by the qualitative MRI assessment in some (Bronen et al., 1995; Kuzniecky et al., 1996), and by the use of different anatomical criteria in the different studies (Quigg et al., 1997; Van Paesschen et al., 1997). We subdivided the hippocampus into head, body and tail according to well-established anatomical landmarks (Duvernoy, 1988). Our finding of a greater atrophy of HH, as compared with HB and HT, is in agreement with pathological data from TLE patients, showing a longitudinal variation in cell density, with a more severe neuronal loss in the anterior hippocampus (Dam, 1980; Babb and Brown, 1987). In autopsy cases from normal controls, Dam (1980) showed that in the anterior hippocampus excitatory dentate granule cell density is greater, while the cell density for the inhibitory hilar neurons is lower. Therefore, in TLE patients, a lack of inhibitory input in the anterior dentate gyrus might lead to a greater excitability and, consequently, to more severe damage in this area (O’Connor et al., 1996). Alternatively, this region may be more susceptible to injury rather than more prone to excitability.

**Distribution of atrophy within the parahippocampal region**

Insauti et al. (1998b) published a comprehensive work on the MRI volumetric analysis of the human entorhinal, perirhinal and temporo-polar cortices based on gross anatomical and cytoarchitectonic borders of these structures on histological sections in normal controls. Although a certain degree of anatomical variation exists in medial temporal lobe structures (Insauti et al., 1998b), the correspondence between volume reduction in the parahippocampal region observed in the
lobe seizures (Scharfman et al., 1998a, 1998b, 2000; Jutila et al., 2001) and neuropathological findings provides support for the validity of the MRI-based volumetric analysis. Our values in normal controls are generally in agreement with those given in previously published protocols (Insausti et al., 1998b; Jutila et al., 2001). The variability of the measurement (percentage standard deviation) in our protocol is however lower. This may be due to the use of higher resolution MRIs (1 mm thick MRI slices versus 2 mm thick slices) in our study, as well as the simultaneous viewing of coronal, axial and sagittal sections, which allows for a more accurate definition of the boundaries. Furthermore, in our data, the inter-individual variability of the parahippocampal volumes is comparable with that of the hippocampus.

In our group of TLE patients, atrophy was not distributed evenly within the parahippocampal region. The EC was more severely affected than the PC. The mean volume of PPC in TLE patients was not statistically different from that of normal controls. However, the individual analysis showed that in 16% of patients, a unilateral atrophy of the PPC was present ipsilateral to the seizure focus. This is the first MRI evidence for the presence of damage in the PPC in TLE.

Pathological changes of the parahippocampal region have not been studied extensively in TLE, mainly because of the inadequacy of the surgically resected specimens usually used for routine examinations (Braak and Braak, 1992). However, in two recent studies performed on en bloc resections of the temporal lobe, pathological changes of the EC were shown in TLE patients (Du et al., 1993), even in the absence of hippocampal sclerosis (Yilmazer-Hanke et al., 2000).

Our finding of a more severe atrophy of the EC concurs with the view that this structure plays a major role in the pathogenesis of TLE. The EC has been shown to contribute to the development and maintenance of epileptiform activity in the temporal lobe (Collins et al., 1983; Jones and Lambert, 1990; Jones and Heinemann, 1991; Pare et al., 1992; Stringer and Lothman, 1992; Du et al., 1995; Avoli et al., 1996; Bear et al., 1996; Federico and Macvicar, 1996; Bragin et al., 1997). In vitro studies of focal epileptogenesis in combined hippocampal–entorhinal slices have demonstrated that the EC possesses an intrinsic capacity to generate epileptiform discharges (Bear and Lothman, 1993). Damage to the EC may contribute to long-lasting changes in its excitability, and may therefore play a primary role in the genesis of temporal lobe seizures (Scharfman et al., 1998). The more severe damage of the EC demonstrated in vivo in our patients with TLE may be due to its remarkable hyperexcitability (Bear et al., 1996; Dickson and Alonso, 1997; Scharfman et al., 1998). This hyperexcitability could also explain our previous observation of EC atrophy in patients with normal hippocampal volumes (Berasconi et al., 2001).

We found a significant atrophy of the PC ipsilateral to the seizure focus in 44% of patients, confirming our preliminary published data (Berasconi et al., 2000). In a recent volumetric study of 25 patients with intractable TLE, the mean volume of the PC did not differ from that of normal controls, but there was a significant asymmetry in patients, with the ipsilateral PC being smaller than the contralateral one (Jutila et al., 2001). There is also evidence for the involvement of the PC in temporal lobe epileptogenesis in animal models of TLE. The PC has been demonstrated to play a role in the convulsive generalization of limbic seizures (Kelly and McIntyre, 1996; McIntyre and Kelly, 2000), and a recent study on an in vitro animal model of seizure spread has shown a direct connection between the hippocampus and PC (Stoop and Pralong, 2000).

**Relationship between hippocampal and parahippocampal region atrophy**

It may be possible to explain the pattern of atrophy within the mesial temporal lobe, predominantly the anterior hippocampus and the EC, by a disruption of entorhinal–hippocampal connections. It is conceivable that preferential anatomical connections between the hippocampus and the EC establish avenues of electrical spread that lead to the severe neuronal loss and atrophy of these structures. The EC plays a pivotal role in the parahippocampal region because it receives most of its cortical inputs from the PC and PPC, and, in turn, gives rise to most of the cortical input to the dentate gyrus, CA1–CA3 fields and the subiculum (Witter and Amaral, 1991). The densest projection along the rostrocaudal axis of the hippocampus is at the transition from the uncal hippocampus, which coincides with the transition of the HH to its HB.

Another explanation for more prominent atrophy of the anterior hippocampus and EC could lie in the distribution of neurotransmitters, GABA in particular, in this region. Parvalbumin is among the calcium-binding proteins that react to GABA as a neurotransmitter (Braak et al., 1991). Immunohistochemical studies in humans have shown that the part of the EC projecting to the uncus contains fewer parvalbumin-immunoreactive neurons and fibres than the part of the EC projecting to the body and tail of the hippocampus. Since a deficit of GABAergic inhibition may underlie some forms of epilepsy (Roberts, 1984), one can speculate that a deficit of GABAergic neurons in the rostral EC, and consequently in the anterior hippocampus, may be responsible for the greater vulnerability of this region to injury.

**Implications for temporal lobe surgery**

Several surgical approaches have been proposed for the treatment of intractable seizures in TLE. These consist of neocortical removal only (Hardiman et al., 1988), anterior temporal lobectomy with a variable extent of amygdalo-hippocampectomy (Engel et al., 1975; Feindel and Rasmussen, 1991; Goldring et al., 1992; Connelly, 1995) and selective amygdalo-hippocampectomy (Wieser and Yasargil, 1982). Interestingly, the type of surgery does not seem to influence surgical outcome, and seizure freedom is reached in the same proportion of patients independently of the type of surgery (Wieser et al., 1990; Arruda et al., 1996).
It is possible that the common denominator in all these procedures might be the excision of the EC. However, it is not known whether the excision of the EC alone would be sufficient to attain seizure freedom. Further studies are needed to assess the relationship between seizure freedom and the extent of resection of different mesial temporal structures.

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**References**


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