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Seizure 2000; **9**: 208–215
doi: 10.1053/seiz.1999.0373, available online at http://www.idealibrary.com on IDEAL

Quantitative MRI volumetry of the entorhinal cortex in temporal lobe epilepsy

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The entorhinal cortex (Brodmann's area 28) is located at the anterior aspect of the parahippocampal gyrus ventral to the amygdala and the hippocampus. It is reciprocally interconnected with the hippocampus via glutamatergic pathways. We investigated whether the entorhinal cortex is damaged in human temporal lobe epilepsy (TLE). The volume of the entorhinal cortex was measured using magnetic resonance imaging (MRI) in 36 patients with cryptogenic TLE and in 21 controls. The mean volumes of the entorhinal cortex on the focal side did not differ from controls. In 11 of 36 patients, however, the entorhinal cortex volume was reduced by 25%. Entorhinal volume correlated with hippocampal volume in TLE (ipsilaterally, $r = 0.454$, $P < 0.01$; contralaterally, $r = 0.340$, $P < 0.05$). Further, 64% of patients with 25% entorhinal cortex damage had ipsilateral hippocampal atrophy. On the other hand, right focal TLE patients with hippocampal atrophy had a 19% volume reduction of the ipsilateral entorhinal cortex ($P < 0.05$). The volume of the entorhinal cortex correlated with the duration of TLE ($r = -0.335$, $P < 0.05$). The present study indicates that the entorhinal cortex might be damaged in a subpopulation of patients with cryptogenic TLE. In most cases, volume reduction was associated with hippocampal damage. These data suggest that entorhinal damage contributes to the symptomatology in TLE.

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Key words: amygdala; cryptogenic; hippocampus; memory; MRI volumetry; seizure.

INTRODUCTION

The medial temporal lobe is composed of the hippocampus, amygdala, and the surrounding cortex, including the entorhinal, perirhinal, and parahippocampal cortices[1]. These areas are interconnected by a myriad of topographically organized connections that function in concert to guide a variety of complex behaviors. These behaviors include the formation of emotional responses to sensory stimuli and memories associated with emotional experiences or everyday facts and events[2]. Hippocampal and amygdaloid damage have been studied extensively in patients with chronic temporal lobe epilepsy (TLE)[3–7]. There are few studies of other functionally distinct areas of the human medial temporal cortex. These demonstrate that damage in the entorhinal cortex is associated with an increased risk for seizure generation in experimental models[8,9]. Further, as a link between the hippocampus and the rest of the cortex, the entorhinal cortex is in a position to gate seizure propagation from the hippocampus to extrahippocampal areas[10–12]. Consistent with animal studies, depth-electrode recordings in TLE patients indicate that the entorhinal cortex contributes to a rapid spread of seizure activity to and from the hippocampus[13–15].

The entorhinal cortex (Brodmann's area 28) is located in the anterior aspect of the parahippocampal gyrus ventral to the amygdala and the hippocampus[1]. Two previous histologic studies have reported neuronal loss in the entorhinal cortex in patients who underwent surgery for refractory seizures[16,17]. Damage was most pronounced in layers II and III of the rostromedial entorhinal cortex[16,17]. This area is reciprocally connected via glutamatergic pathways with the rostral half of hippocampus[1,18,19], which is damaged in 60–70% of patients with refractory TLE[7].

Because the entorhinal cortex has heavy reciprocal connections with the hippocampus and surrounding cortex via glutamatergic pathways[1,18,19], we hypothesized that the generation and spread of seizure activ-

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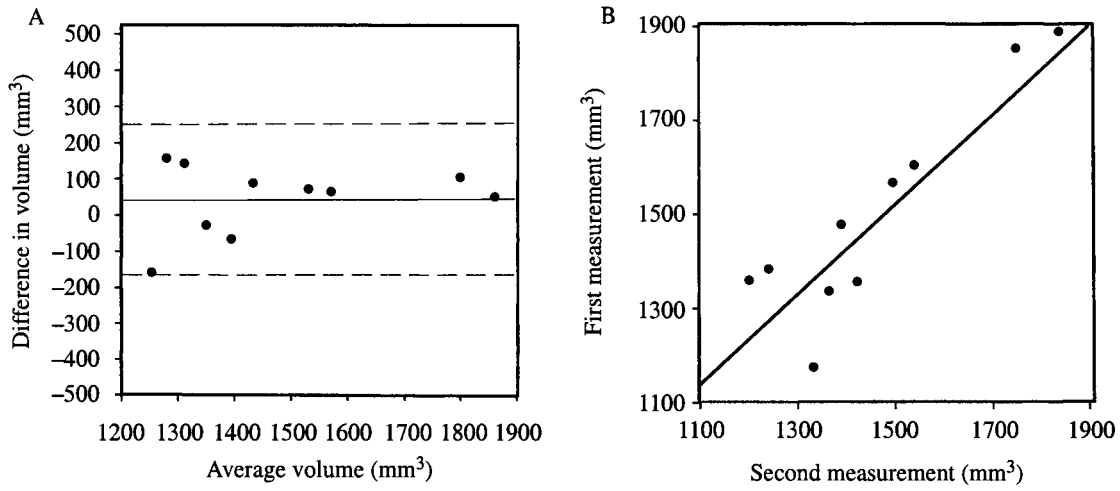

Fig. 1: a, A scatter plot showing the repeatability of the volumetric measurements of the entorhinal cortices according to Bland and Altman[23] in controls. The limits of agreement (dashed lines) between the first and second measurements are expressed as the mean difference in volume [volume in the first measurement − volume in the second measurement (mm^3) ± 2 SD]. b, Correlation between the first and the second measurement of the volume of the entorhinal cortex. Abbreviation: SD, standard deviation.

ity relates not only to damage in the hippocampus but also to the entorhinal cortex in human TLE. To test this hypothesis, we investigated whether the volume of the entorhinal cortex is reduced in chronic TLE. Second, we determined whether the entorhinal damage is isolated or associated with hippocampal and/or amygdaloid damage. The volume of the entorhinal cortex was assessed in 36 patients with cryptogenic TLE and in 21 controls using a recently developed magnetic resonance imaging (MRI)-volumetric protocol[20]. Patients with cryptogenic TLE were chosen to minimize the contribution of various etiologic factors to the entorhinal damage. While we were completing the present study, Bernasconi *et al.*[21] reported that drug-refractory TLE patients evaluated for epilepsy surgery had a volume reduction in the entorhinal cortex.

MATERIALS AND METHODS

Study population

Controls. The control population included 21 healthy individuals (10 males, 11 females) with a mean age of 32 ± 9 (range 21–52) years. All controls were interviewed in order to exclude those with neurologic diseases.

Patients with TLE. Thirty-six patients (16 males, 20 females; age 37±13 years, range 16–63 years) with chronic cryptogenic TLE were included in the study. The TLE patients were referred to the Department of Neurology at the Kuopio University Hospital and consecutively examined using MRI during the years 1993–1996. The majority of the patients had visited a neurologist at the epilepsy unit of the Department of Neurology regularly since the time of their epilepsy diagnosis. Moreover, at the time of the MRI, all patients were examined by a neurologist at the epilepsy unit. Diagnosis and the etiologic factors of TLE were evaluated on the basis of medical history, neurologic examination, electroencephalography (EEG), and qualitative MRI. Seizure symptomatology and non-invasive EEG data were used to determine the laterality of TLE.

Seventeen patients had the seizure focus in the left temporal lobe (8 males, 9 females; age 40 ± 12, range 24–60 years) and 19 patients had the seizure focus in the right temporal lobe (8 males, 11 females; age 36 ± 14, range 16–63 years). Patients had experienced TLE symptoms for a median of 20 (range 7–58) years. There were no known etiologic factors (vascular, hypoxic, traumatic, infectious, or congenital) that might have contributed to damage in the medial temporal lobe. Patients with prolonged febrile seizures were excluded.

To calculate the number of partial and secondary generalized seizures each patient experienced during their lifetime, we carried out an extensive search for the lifetime hospital records of each patient. The total number of seizures was calculated from these hospital notes. In Finland, the maintenance of hospital services for patients with epilepsy is the responsibility of the community, and the Finnish health care system makes it possible with the permission of the patient to obtain copies of hospital records from community hospitals.

MR imaging

The patients and controls were scanned with a 1.5 T imager Magnetom (Siemens; Erlangen, Germany) using a standard head coil and a tilted coronal 3D magnetization-prepared rapid acquisition gradient-echo (MP-RAGE) sequence with parameters of 10/4/1 (TR/TE/excitations); inversion time 250; flip angle 12°; field of view 250 mm; matrix 256 × 192. This resulted in 128 contiguous T1-weighted images with a slice thickness of 2 mm.

Volumetry of the entorhinal cortex. The same observer (T.S.) measured the volumes of all entorhinal cortices using a recently described histology-based method[20]. At the time of the volumetric measurements, the investigator was unaware of the side of the seizure focus.

The entorhinal cortex (Brodmann's area 28) lies adjacent to the hippocampus and the amygdala and forms the major portion of the anterior parahippocampal gyrus[1]. The entorhinal cortex borders the periamygdaloid cortex medially and the perirhinal cortex laterally[1]. The caudal limit of the entorhinal and perirhinal cortices is contiguous with the posterior parahippocampal cortex[1] (areas TH and TF by Bailey and von Bonin[22]). In order to define the borders of the entorhinal cortex on MR images[20], the following procedure was performed. A midsagittal plane was drawn through the anterior and posterior commissures, and coronal images perpendicular to this plane covering the entire rostrocaudal length of the entorhinal cortex were obtained and reconstructed into 2 mm thick contiguous slices. For volumetry, the images were magnified and interpolated fourfold, which resulted in an effective pixel size of 0.25 mm. After identification of the sulci and gyri, as well as the rostrocaudal level of each image relative to the limen insula, the outline of the entorhinal cortex was manually traced using a trackball-driven cursor on successive MR images as previously described[20]. The volume of the entorhinal cortex was then calculated with software developed in-house for a standard work console.

The intra-observer variability, expressed as the mean of the coefficient of variation of each control, was 4.9%. Figure 1 shows the repeatability of the volumetric measurements of the entorhinal cortices according to Bland and Altman[23]. The mean difference between the two measurements was near zero and the limits of agreement were below the volume considered to be a marked volume reduction (i.e. $\geq$2 standard deviations (SD) from the mean of the controls, 424 mm^3 for the entorhinal cortex).

Volumetry of the hippocampus and the amygdala. The method used for the volumetry of the hippocampus and the amygdala has been previously described in detail[24]. The intra-observer variability for hippocampal volumes was 6.8% and for amygdaloid volumes, 8.9%.

Statistical analyses

To correct the volumes of the hippocampus, amygdala, and entorhinal cortex for inter-individual differences in head size, we used the equation determined by Cendes *et al.*[25] modified as previously described[26,27] [instead of brain volume, we used the brain area which correlates with the brain volume ($r = 0.67$, $P < 0.001$, $n = 20$)]. The mean brain area measured at the level of the anterior commissure of the controls was divided by the corresponding brain area of the patient. Thereafter, this ratio was multiplied by the measured volume of the hippocampus, amygdala, or entorhinal cortex. To examine the degree of asymmetry in the volumes of the hippocampus, amygdala, and entorhinal cortex, we calculated the asymmetry ratio according to Bernasconi *et al.*[21] as follows: Asymmetry % $= [100 * (R - L)]/[(R + L)/2]$ in which R refers to the right side and L to the left.

The normalized data were analysed with SPSS-Win V7.5 software (Chicago, IL). The following comparisons were made: (1) controls with all patients and (2) controls with patient groups divided according to the side of seizure focus. Sixteen of 36 TLE patients had a marked hippocampal volume reduction [i.e. volume of the hippocampus was $\geq$2 SD below the mean volume in controls]. Therefore, we also compared controls with left focus TLE patients with or without $\geq$2 SD hippocampal damage, and controls with right focus TLE patients with or without $\geq$2 SD hippocampal damage. The Kruskal–Wallis test was used to compare the mean volumes between the study groups. Differences between the controls and patient groups were determined using the Mann–Whitney U-test with the Bonferroni correction. Non-parametric analyses were performed because the variables were not normally distributed in all the groups and not equal in variance. Correlations were calculated using the two-tailed Pearson's correlation test. A P-value of <0.05 was considered to be statistically significant.

RESULTS

Entorhinal volumetry

Entorhinal volumes are shown in Table 1 and hippocampal and amygdaloid volumes are shown in Table 2. The Kruskal–Wallis test indicated no significant

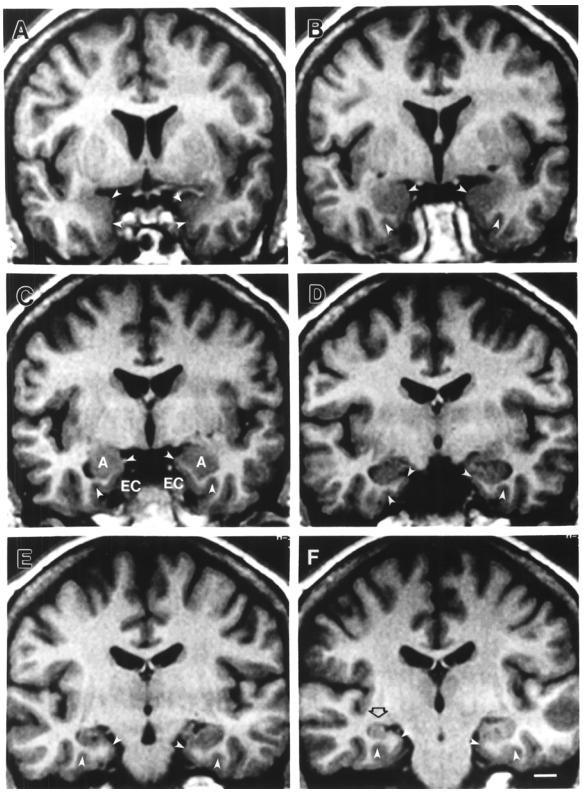

Fig. 2: Coronal MR images from six rostrocaudal levels of the entorhinal cortex (indicated with arrowheads) taken from a 28-year-old female with chronic cryptogenic TLE and seizure focus on the right. Panel A shows the most rostral level (shows the beginning of the EC[20]) and panel F shows the most caudal level. The volume of the ipsilateral entorhinal cortex was 69%, hippocampus 49% (indicated with an open arrow in panel F), and amygdala 87% of that in controls. Abbreviations: A, amygdala; EC, entorhinal cortex. Scale bar 10 mm.

differences in the volumes of the left entorhinal cortex when patients with left focus, right focus, and controls were compared. Nor was there any difference in the mean volume of the right entorhinal cortex between the study groups. Also, the asymmetry index for the entorhinal cortex did not differ from that in controls.

Because no differences were found in the mean volumes between the groups, we assessed whether the magnitude of entorhinal damage was reduced in any subject individually. Two of 36 patients had $\geq$2 SD volume reduction of the entorhinal cortex (i.e. at least a 31% volume reduction on the left side or 41% on the right side). In 11 of 36 patients, entorhinal volume was reduced at least by 25% (see an example in Fig. 2). The volume reduction was ipsilateral in 5 of these 11 patients, bilateral in 3, and contralateral in 3.

Further analysis indicated significant differences in the entorhinal volume when the occurrence of hippocampal damage ($\geq$2 SD volume reduction compared to controls) was taken into account. In patients with TLE, the entorhinal volume correlated with the hippocampal volume ipsilaterally ($n = 36$, $r = 0.454$, $P < 0.01$; Fig. 3a) and contralaterally ($n = 36$, $r = 0.340$, $P < 0.05$). Overall, 7 of 16 patients with hippocampal damage had $\geq$25% volume decrease in the ipsilateral entorhinal cortex. In right focus TLE patients with ipsilateral hippocampal damage, the mean volume of the ipsilateral entorhinal cortex was reduced by 19% compared with controls ($P < 0.05$). Also, the left focus TLE patients with left hippocampal damage had a 16% volume reduction of the ipsilateral entorhinal cortex compared with controls but the difference was not significant ($P = 0.0936$). Of 11 patients with $\geq$25% entorhinal damage, 7 had ipsilateral hippocampal damage.

Analysis of the coexistence of the entorhinal and amygdaloid damage ($\geq$2 SD volume reduction) indicated that the entorhinal and amygdaloid volumes correlated ipsilaterally ($n = 36$, $r = 0.346$, $P < 0.05$; Fig. 3b) but not contralaterally. Of 11 patients with $\geq$25% entorhinal damage, only 2 had ipsilateral amygdaloid damage. Of 4 patients with amygdaloid damage, 3 had $\geq$25% volume reduction in the ipsilateral entorhinal cortex.

The hippocampal asymmetry index differed between patients with a left or right focus ($P < 0.01$; Table 2). There was no difference in the asymmetry index for the amygdala in the present study population.

Correlation of the volume of the entorhinal cortex with duration of TLE and number of seizures

In all patients, the ipsilateral entorhinal volume correlated inversely with the duration of TLE ($n = 36$, $r = -0.335$, $P < 0.05$; Fig. 4a). There were no differences, however, in the mean entorhinal volume between the patient groups with TLE onset $\leq$5 and >5 years of age ($n = 11$, ipsilateral volume 1180 ± 224 mm^3, contralateral volume 1345 ± 251 mm^3; $n = 25$, ipsilateral volume 1289 ± 238 mm^3, contralateral volume 1265 ± 251 mm^3, respectively). There was no correlation between the estimated seizure number (partial, secondarily generalized, or all seizures) and the entorhinal volume ipsilaterally (Fig. 4b) or contralaterally.

DISCUSSION

The present study investigated the appearance of entorhinal damage in patients with cryptogenic TLE. While we were completing the study, Bernasconi *et al.*[21] reported a reduction in the mean volume of the entorhinal cortex ipsilateral to seizure focus in patients who were evaluated for epilepsy surgery. We did not, however, find a difference in the mean entorhinal vol-

Table 1: Volumes (mm^3) of the left and right entorhinal cortex in patients with cryptogenic temporal lobe epilepsy and controls.

	Left EC	Damage %	Right EC	Damage %	Asymmetry %
Controls (21)	1369 ± 210		1417 ± 289		2.6 ± 13.1
All patients (36)	1234 ± 212[a]	10	1310 ± 267	8	5.4 ± 16.2
Left TLE (17)	1255 ± 240	8	1371 ± 291	3	8.3 ± 14.0
without HC damage (10)	1330 ± 263	3	1381 ± 287	3	3.7 ± 10.5
with HC damage (7)	1148 ± 166	16	1358 ± 319	4	14.9 ± 16.5
Right TLE (19)	1216 ± 187	11	1256 ± 239	11	2.7 ± 18.0
without HC damage (10)	1230 ± 185	10	1358 ± 262	4	9.4 ± 8.7
with HC damage (9)	1200 ± 199	12	1141 ± 152[a]	19	-4.4 ± 22.8

Normalized values are shown as mean $\pm$ standard deviation of the mean. Damage % shows the percentage of volume reduction below the mean in controls. The asymmetry ratios are expressed as percentages in the table. The number of patients is in parenthesis. Statistical significances were calculated with Kruskal–Wallis and Mann–Whitney analyses: [a] $P < 0.05$ compared with controls. Abbreviations: EC, entorhinal cortex; HC, hippocampus.

Table 2: Volumes (mm^3) of the left and right hippocampus and amygdala in patients with cryptogenic temporal lobe epilepsy and controls.

	Left HC	Right HC	Asymmetry %	Left AMY	Right AMY	Asymmetry %
Controls (21)	3439 ± 357	3687 ± 460	6.7 ± 8.0	2528 ± 397	2358 ± 265	-6.3 ± 10.6
Patients (36)	3155 ± 840	3291 ± 912	3.7 ± 32.8	2422 ± 317	2352 ± 380	-3.3 ± 14.6
Left TLE (17)	2851 ± 1005	3491 ± 744	23.4 ± 26.0	2434 ± 364	2406 ± 350	-1.0 ± 16.0
Right TLE (19)	3426 ± 557	3113 ± 1027	-13.8 ± 28.2[a]	2410 ± 277	2303 ± 409	-5.4 ± 13.5

Normalized values are shown as mean $\pm$ standard deviation of the mean. The number of patients is in parenthesis. The asymmetry ratios are expressed as percentages in the table. Statistical significances were calculated with Kruskall–Wallis and Mann–Whitney analyses. [a] $P < 0.01$ compared with left TLE patients. Abbreviations: AMY, amygdala; HC, hippocampus.

ume ipsilaterally. This discrepancy probably relates to less severe TLE and brain damage in our patient group compared with that of Bernasconi *et al.*[21]. This assumption is supported by the finding that the asymmetry indices for the hippocampus, amygdala, and the entorhinal cortex were less abnormal in the present study than in the Montreal study[21]. The volume of the entorhinal cortex was reduced in a subgroup of patients with cryptogenic TLE, however, in the present study. Second, in most cases, entorhinal volume reduction is associated with hippocampal damage. Finally, the severity of entorhinal damage correlates with the duration of TLE.

Factors associated with entorhinal damage in TLE

None of the patients included in the present study had any identifiable etiologic factors in their medical history that could have been associated with entorhinal damage. In a previous histologic study by Du *et al.*[16], one of four patients with entorhinal damage was born prematurely and one had febrile convulsive status epilepticus at the age of 9 months. The two other patients, however, had no definitive etiologic factors for TLE. In the recent MRI study of Bernasconi *et al.*[21], the etiologies of TLE in patients with entorhinal damage were not specified. Taken together, histologic data together with the present MRI observations indicate that entorhinal damage can occur even without any known previous brain insult.

In 45% of cases with a decrease in entorhinal volume of $\geq$25%, the reduction was exclusively ipsilateral to seizure focus. In 27%, it was contralateral to seizure focus, and in 27%, bilateral. This suggests that the appearance of entorhinal damage has limited value as a lateralizing measure. Otherwise, a majority (64%) of patients with $\geq$25% entorhinal damage had hippocampal atrophy. Further, in 44% of cases, hippocampal damage was associated with entorhinal damage. Therefore, in a substantial percentage of cases with TLE, damage occurs in more than one structure of the medial temporal lobe.

Another factor associated with entorhinal damage was the duration of epilepsy. Unlike hippocampal damage[28], however, entorhinal damage was not any more severe in patients with an onset of epilepsy before 5 years of age than in patients with later onset of epilepsy. Also, there was not such a clear association between the lifetime seizure number and entorhinal damage as observed previously with hippocampal damage. This suggests that the hippocampus is a more sensitive structure for seizure-induced damage than is the entorhinal cortex. Alternatively, it might be more difficult to detect entorhinal than hippocampal damage when the total volume of the structure is measured.

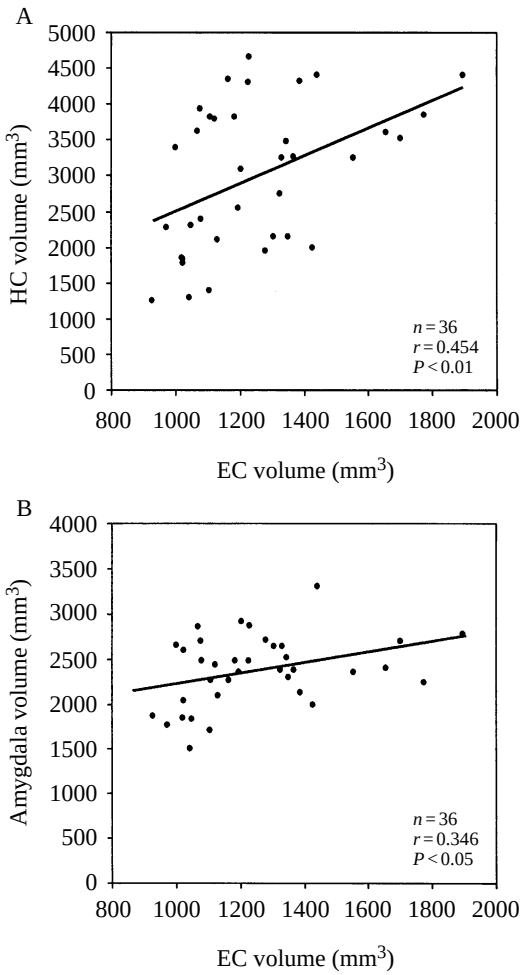

Fig. 3: a, A scatter plot showing the correlation between the volumes of the entorhinal cortex and the hippocampus ipsilateral to the seizure focus in all patients. b, A scatter plot showing the volume of the entorhinal cortex in relation to the volume of the amygdala ipsilaterally. In controls, there was no correlation between the volume of the entorhinal cortex and that of the hippocampus or the amygdala on the left. On the right, however, the entorhinal volume correlated with the hippocampal ($n = 21$, $r = 0.585$, $P < 0.01$), but not with the amygdaloid volumes. Abbreviations: EC, entorhinal cortex; HC, hippocampus; n, number of patients; r, correlation coefficient (Pearson).

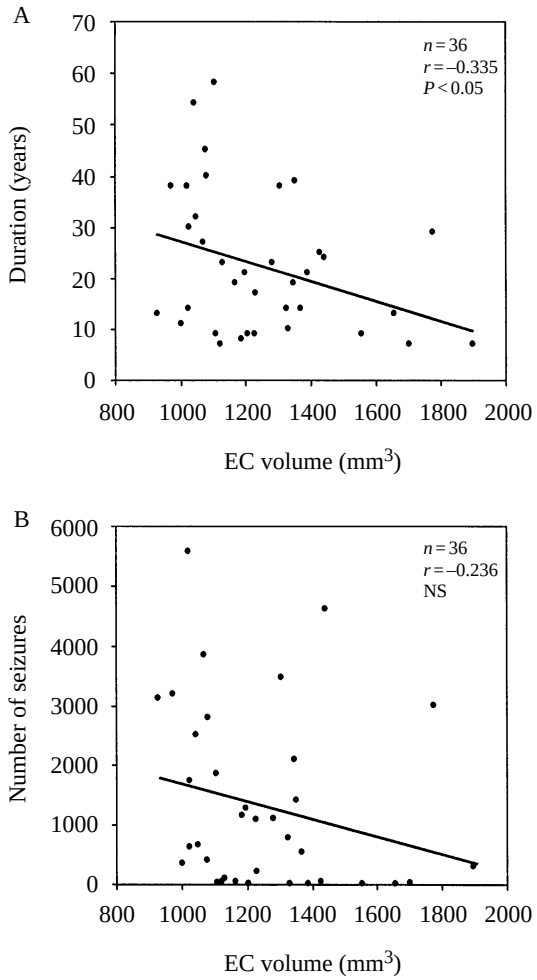

Fig. 4: a, A scatter plot showing the correlation between the volume of the entorhinal cortex ipsilateral to the seizure focus and the duration of TLE. After logarithmic transformation of the volume values: $r = -0.338$, $P < 0.05$. b, A scatter plot showing the correlation between the volume of the ipsilateral entorhinal cortex and the lifetime number of partial seizures. After logarithmic transformation of the volume values: $r = -0.278$, $P > 0.05$. Abbreviations: EC, entorhinal cortex; n, number of patients; NS, not significant; r, correlation coefficient (Pearson).

Appearance of entorhinal damage

The largest reduction in volume of the entorhinal cortex was 42%. Considering that the human entorhinal cortex is cytoarchitectonically, chemoarchitectonically, and connectionally a heterogeneous region that can be divided into eight subfields[19, 29, 30], the question arises: are all parts of the entorhinal cortex damaged to the same degree? Two previous histologic studies indicate that layers II and III of the rostromedial entorhinal cortex (EO and ER subfields) have remarkable neuronal loss in patients operated on for drug-refractory seizures[16, 17]. So far, surgically resected tissue for histologic analysis was obtained from two subjects included in the present MRI study. Both cases had reduced entorhinal volume ipsilaterally and histologic analysis revealed neuronal loss in layer III of the ER subfield (data not shown). Given that layers II and III contain more than 50% of the 6.9 million entorhinal neurons[31], it is reasonable to assume that the entorhinal volume reduction in TLE reflects neuronal loss in the superficial layers. Otherwise, the EO and ER subfields comprise less than one-third of the total surface area of the human entorhinal cortex (see Fig. 2 in Ref.[19]). Therefore, it remains to be determined whether damage to these rostral subfields accounts for the majority of the entorhinal volume reduction observed in the present study, or whether more caudal subfields are involved.

Functional considerations

Most of the current knowledge about the neuropathology and electrophysiology of TLE comes from studies of the hippocampus. Recent imaging studies, however, indicate that volumes of the amygdala (for review, see Pitkänen *et al.*[32]), thalamus[33], mammillary bodies[34], fornix[35], lenticular nucleus[33], and caudate nucleus[33], might be reduced in patients with TLE. Previous histologic studies as well as the recent quantitative MRI study by Bernasconi *et al.*[21] and the present observations indicate that the entorhinal cortex is damaged in a subgroup of patients with TLE. Overall these data emphasize the fact that damage outside the hippocampus has to be taken into account when tracing the structural substrates for the symptomatology of TLE, such as seizure generation or memory impairment.

ACKNOWLEDGEMENTS

This study was financially supported by the Academy of Finland, the Vaajasalo Foundation, and the Sigrid Juselius Foundation.

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