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Rhinal cortex asymmetries in patients with mesial temporal sclerosis

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Summary

Purpose: The rhinal cortex, comprising the entorhinal (ErC) and perirhinal (PrC) cortices, is one component of the limbic system that may be affected in patients with epilepsy and other temporal lobe pathologies. This study extended quantitative examination of the limbic system through development and validation of volumetric protocols to measure the ErC and PrC.
Methods: Volumes were calculated from MRI studies using ANALYZE 7.5 and based on detailed anatomical definitions developed for the study. Subjects were 61 temporal lobe epilepsy patients with mesial temporal sclerosis (MTS: 33 left, 28 right) and 20 neurologically normal controls. Inter-rater reliabilities for the ErC and PrC volume protocols were found to be high (range 0.86—0.92).
Results: Ipsilateral hippocampal volume was reduced in patients with MTS, while contralateral volume did not differ significantly from controls. In the patients, rhinal cortex volumes were reduced as a function of laterality of disease. The pattern of correlations between ErC and PrC differed between disease groups. Hippocampal and rhinal cortex volumes were not significantly correlated. A significant four-way interaction was found between side of MTS, hemisphere, structure and handedness.
Conclusions: This quantitative study demonstrates reliable *in vivo* evidence of morphometric changes in ErC and PrC in a substantial number of patients with unilateral MTS. The relationship observed between handedness, structure and disease status may suggest a role for cerebral dominance in modulating the expression of MTS.

Introduction

The entorhinal (ErC) and perirhinal (PrC) cortices are crucial components in the pathway through which highly processed information from the neocortex

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reaches the hippocampal formation and the amygdala.[1] ErC, PrC as well as the parahippocampal gyrus (PHG) are heavily interconnected.[2] The PrC receives input from the temporal, parietal, occipital, cingulate and insular cortices and is one of the major inputs to the ErC, which then conveys this highly processed information to the hippocampus via the perforant pathway.[3,4] So, while the ErC connects directly to the hippocampus, connections from the PrC and PHG to the hippocampus are indirect, via relays in the ErC.[5,6] The hippocampus is thus the final stage of convergence within the medial temporal lobe.[7]

Progress in the modelling of medial temporal lobe (limbic system) function has been limited by the technological problems associated with characterising deep brain structures *in vivo*.[8] The advent of volumetric magnetic resonance imaging (MRI) analysis provides an opportunity to clarify issues of human temporal lobe structure, by permitting a direct investigation of *in vivo* morphology. MRI volumetry is based on the principle that the response of neurons to disease is gliosis and cell loss.[9] It follows that neural disease may be reflected in the measurement of discrete structures *in vivo*. Indeed, several studies have documented a close correlation between histopathologically determined cell loss and atrophy as measured through hippocampal volume measurements.[10–12] Several research groups now regard volumetric MRI as a surrogate for histological examination of the hippocampus[9,13] and incorporate this technique routinely in their pre-surgical analysis of temporal lobe epilepsy (TLE) patients with mesial temporal (hippocampal) sclerosis (MTS).

The exact limbic structures involved in MTS remain uncertain.[14] Histopathologically, MTS has been defined as cell loss and reactive gliosis in the hippocampus, predominantly in field CA4, but often involving areas CA1 and CA3 and the subiculum.[15] The amygdala and dentate fascia are also often affected.[16] Attempts to define the extent of damage in adjacent structures, namely the ErC and PrC, have been sparse.[17] Recently, one group documented ErC volume reductions ipsilateral to the epileptic focus in MTS patients with hippocampal volume reductions,[18] as well as those with normal hippocampal volume.[19] In the latter study, nine of 22 patients had histopathologically confirmed MTS, but ErC volumes were examined in the group as a whole, making it difficult to draw conclusions about the significance of ErC changes. Salmenpera and colleagues documented ErC changes in a subpopulation of patients with cryptogenic TLE, with mixed pathology, but did not examine MTS patients separately.[20] The involvement of ErC in the generation and propagation of temporal lobe seizures has been documented in animal and human research.[21,22] The PrC, with its close anatomical and functional connections with the ErC, may also show morphometric changes in patients with MTS. Bernasconi and colleagues found PrC abnormalities in two of six TLE patients examined. However, the distributional properties of the volume changes were not discussed.[23]

The present study extends quantitative examination of the limbic system through the development and validation of detailed volumetric protocols to measure the ErC and PrC. The volumetric protocols will then be used to examine relationships in the rhinal cortex in a substantial homogeneous group of TLE patients, all with MTS. The fact that this disorder can result in pathological changes in different mesial temporal structures also provides a unique opportunity to explore relationships between structures of interest, without the confounds of invasive techniques. The proposed research has the potential to improve our understanding of the patterns of limbic pathology in patients with MTS.

Materials and methods

Participants

Subjects were 61 consecutive patients with TLE admitted to the Comprehensive Epilepsy Program at St. Vincent's Hospital, Melbourne. The Commission on Classification and Terminology of the International League Against Epilepsy[24] classifies TLE as a symptomatic form of location-related (focal or partial) epilepsies and syndromes. Patients were identified on the basis of clinical features, interictal scalp/sphenoidal EEG, prolonged EEG-video monitoring and neuropsychological studies. Fifty-nine of the 61 patients have so far proceeded to anterior temporal lobectomy, with histopathological confirmation of MTS in all cases. Till date, two patients have chosen not to proceed with surgery, but results of the above investigations are suggestive of typical MTS. Thirty-three patients had left MTS, 28 had right MTS. Of the patients with left MTS, 14 were male and 19 were female, with a mean age of 37 years (S.D. = 9.4). Nine of the left MTS group were left-handed, 24 were right-handed. Handedness was defined as laterality preference, as assessed by a handedness questionnaire routinely administered by the treating neurologist during initial consultation. The questionnaire is available on request from the first author. For brevity, laterality of preference will be referred to subsequently as handedness. Of the right MTS group, 15 were male and 13 were female, with a mean age of 36 years (S.D. = 11.9). Six of this group were left-handed, 22 were right-

handed. Twenty neurologically normal community volunteers were examined as the control group, taken from a consecutive series of participants in a wider research study being undertaken at St. Vincent's Hospital. The control subjects had no history of head injury or significant medical or psychiatric illness. Ten control subjects were female, 10 were male. Mean age for all control subjects was 34 years (S.D. = 14.8). Handedness data was available on 11 control subjects, 9 of whom were right-handed, 2 were left-handed. There were no significant differences between control and patient groups on age ($F(2,78) = 0.617$, $p = 0.542$) or gender ratio ($\chi^2 = 0.790$, d.f. = 2, $p = 0.674$).

Data collection and imaging analysis of the patients was conducted under approval of the Human Research Ethics Committee of St. Vincent's Hospital, Melbourne.

MR acquisition

The MRI images were acquired pre-surgically for the MTS patients, using a high-resolution 1.5-T scanner (Seimens and G.E. systems). Validation studies have been conducted in the past to evaluate the comparability of the images obtained from the Seimens and G.E. systems. The techniques have been found to be reliable and stable across the two systems.[25] We have also studied 'phantoms' of all available MRI scans at the time of publication and demonstrated the reliability of volume and linear estimates across these volumes. Primary data is available from the authors upon request. Images from control subjects were processed using the G.E. system and patient images were analysed using the Seimens system. A coronal T1 weighted sequence generated 160 contiguous 1.4 mm slices. All images were inspected carefully to exclude other intracranial lesions.

Image processing

Images were transferred to an off-line dedicated workstation for use with an image analysis software program, Analyze 7.5 (Mayo Foundation, Rochester, MN). Using the MRI series, the brain was extracted from the head scan by way of a 3D morphometric technique. The cerebellum and brainstem were then disarticulated from the rest of the cerebrum. The next step involved manually editing of internal grey structures on contiguous sagittal slices throughout the series.

Technique of measurement

Rigid anatomical landmarks were used to define the boundaries on the ErC and PrC cortices (see below). The region of interest measurements were performed by an operator (CM), who was blind to the side of MTS. Reliability of the protocol was then examined by a second rater (MC), who measured ErC and PrC on the same 20 MRI studies, blinded to the results of the first rater. The inter-rater reliabilities (Pearson product—moment correlations) for left and right ErC were 0.86 and 0.90, respectively, and for left and right PrC, inter-rater reliabilities were 0.92 and 0.91, respectively. Anatomical specifications for the borders of ErC and PrC are defined at three levels moving through the structures from anterior to posterior. ErC and PrC volumes were traced manually according to the defined protocol, using a tracker-ball driven cursor. Area and volume were automatically calculated by pixel counting. Hippocampal volumes for patient and control subjects were measured previously by MC using published protocols,[10,26] as part of routine pre-surgical evaluation in the MTS patients and the wider research study in the control group.

Histological and gross anatomical definitions

The ErC and PrC denote a region of cortex in the ventromedial part of the temporal lobe, extending from the tip of the temporal pole to the posterior limit of the uncus.[8] The exact location of ErC and PrC in humans, and the distinction between the two structures, remains somewhat controversial.[27] The human ErC corresponds to Brodmann's areas 28 and 34. The rostral limit of ErC is associated with the appearance of the limen insulae.[17,28] Medially, the ErC forms the surface of the gyrus ambiens. Laterally, the PrC borders the ErC along the medial bank of the collateral sulcus, although the exact transition between ErC and PrC is not well defined.[18] At the posterior limit of ErC and PrC, the ErC is continuous with the posterior PHG.[8] No gross anatomical feature defines the point at which ErC becomes PHG, and different studies include varying degrees of PHG in the measurement of ErC.[17,18] The appearance of the intralimbic gyrus has been used as an easily identifiable marker for the posterior limit of ErC, at the transition between ErC and PHG.[17]

The PrC comprises two cytoarchitectonically distinct areas (Brodmann's areas 35 and 36) and is situated in the lateral bank of the rhinal sulcus and in the laterally adjacent cortex.[4] On the ventral surface of the brain, the PrC includes much of the inferotemporal gyrus, known as the band of cortex between the anterior middle temporal sulcus and the rhinal sulcus. Anteriorly, the rostral tip of PrC lies a few millimetres anterior to the limen insulae.[17] At this point, PrC is continuous with the

rostral-most portion of the temporal pole (Brodman's area 38).[17] The caudal limit of the PrC has been defined as the level of the intralimbic gyrus.[17] For the purposes of the present study, volumetric protocols were defined according to the cortical topographic landmarks on MRI that best approximated the cytoarchitectual boundaries described (see next section).

It should be noted that the collateral sulcus is highly variable in shape and length and may be asymmetric between left and right hemispheres.[28] Previous studies have documented greater variability among ErC and PrC measurements than for hippocampal and amygdala measurements.[26,29] The variability in measurements appears to be a reflection of biological variation, rather than measurement error, as similar variation was obtained when measurements were taken directly from histological sections.[28]

Definitions of borders of entorhinal and perirhinal cortices on MR images

A summary of the volumetric protocols is detailed in Table 1.

For the purposes of the present study, the crest of the medial bank of the collateral sulcus was regarded as an easily reproducible marker for the border between ErC and PrC. Anteriorly, the limit of ErC and PrC was defined as the last section where white matter is clearly continuous between the frontal and temporal lobes, immediately posterior to the limen insulae (Fig. 1A). At this point, ErC is defined according to the following boundaries. The rostral border is formed by a line traced from the apex of the gyrus ambiens (most medial point of cortex) to the nearest grey–white interface. The caudal limit is defined by a line drawn from the medial crest of the collateral sulcus to the nearest grey–white interface.

The lateral limit is the border between white matter and cortex. The medial boundary is the outer limit of the cortex. The borders of PrC at this level are as follows. The rostral limit is the caudal limit of ErC, that is, a line drawn from the medial crest of the collateral sulcus to the nearest grey–white junction. The area nominated as PrC extends caudally to the lateral crest of the collateral sulcus, where a line is drawn to the nearest grey–white interface. Here the PrC borders with the inferior temporal gyrus (area T3).

The borders of ErC and PrC remain the same until the emergence of the uncal fissure, where the hippocampus and amygdala are easily discernable (Fig. IB). At this point, the rostral border of ErC is defined as a line drawn from the lateral crest of the uncal fissure to the nearest grey–white interface. All other anatomical boundaries remain the same. The posterior limit of ErC and PrC is defined as the last slice in which the intralimbic gyrus is visible (Fig. 1C). At this point ErC is continuous with the PHG.

Statistical analysis

Data was analysed using SPSS version 10.0 software for Windows.[30] A series of analysis of variance (ANOVA) procedures was used to compare the control group, left- and right-sided MTS patients on hippocampal, ErC and PrC volumes. Pearson corre-

Table 1 Definition of neuroanatomical boundaries for entorhinal cortex and perirhinal cortex

	Entorhinal cortex	Perirhinal cortex
Anterior limit: immediately posterior to the limen insulae		
Rostral	Line drawn from the apex of the gyrus ambiens to nearest grey–white border	Line drawn from the medial crest of collateral sulcus to the nearest grey–white interface
Caudal	Line drawn from medial crest of collateral sulcus to nearest grey–white interface	Line drawn from the lateral crest of collateral sulcus to the nearest grey–white junction
Lateral	Border between white matter and cortex	Border between white matter and cortex
Medial	Outer limit of cortex	Outer limit of cortex
Uncal fissure: emergence of the uncal fissure		
Rostral	Line drawn from the lateral crest of the uncal fissure to the nearest grey–white interface	Unchanged
Caudal	Unchanged	Unchanged
Lateral	Unchanged	Unchanged
Medial	Unchanged	Unchanged
Posterior limit: last slice in which intralimbic gyrus is visible		
Borders remain the same for all structures as defined at the emergence of the uncal fissure		

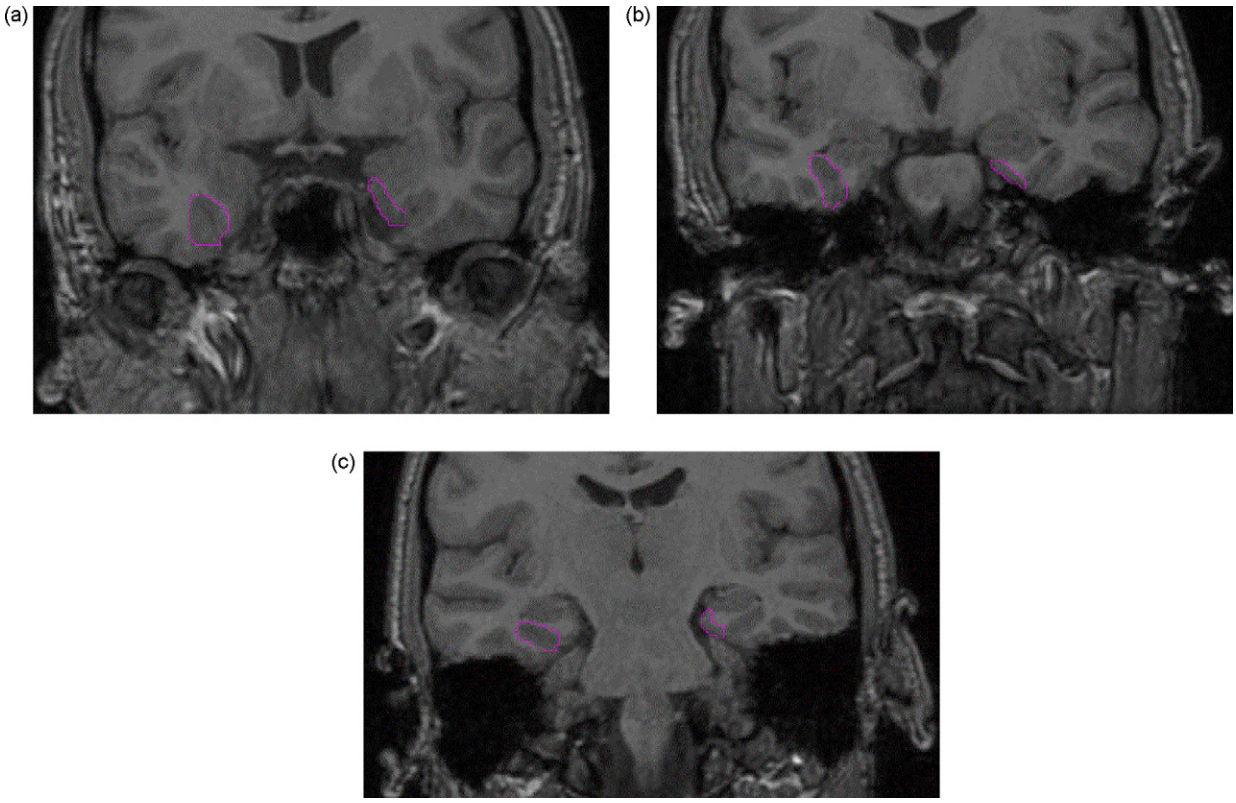

Figure 1 (A) Anterior limit of entorhinal and perirhinal cortex boundary. This figure and figures B and C (see below) depict oblique coronal images subvolumed to focus on the temporal region. Tracings of the left hemisphere (right side of image) indicate entorhinal cortex region-of-interest protocols. Tracings on the right hemisphere (left side of image) indicate perirhinal cortex boundaries. (B) Entorhinal and perirhinal cortex boundaries at the emergence of the uncal fissure. (C) Posterior limit of entorhinal and perirhinal cortex boundaries.

lations were calculated to compare volumes of each structure in the patient and control groups. Paired-samples *t*-tests were used to examine hemispheric differences for hippocampal, ErC and PrC volumes in the control group. The effects of handedness, side of disease, hemisphere, gender and structure on volume in patients were examined with repeated-measures ANOVA procedures. Tukey's post hoc contrasts with Bonferroni-type corrections were used to examine differences between means. The effect of age and gender on hippocampal, ErC and PrC volume was investigated using Pearson correlations to explore age effects, and *t*-tests to examine gender differences. Assumptions underlying all statistical tests were examined, including homogeneity of variance and homoscadasticity of the variance—covariance matrices for univariate and repeated measures ANOVAs, respectively. Normality assumptions were examined for t-tests and Pearson correlations. In all cases assumptions of analyses were met with one specific exception, as indicated below. Magnitude of statistical effects are reported for ANOVAs as η^2, for *t*-tests as Cohen's *d*, and for Pearson's correlations as Cohen's *r*.[31]

Results

Descriptive statistics (mean, standard deviation) for hippocampal, ErC and PrC volumes in the control, left- and right-sided MTS groups are listed in Table 2.

A series of separate two-way ANOVAs was performed to analyse volume differences between the control group and MTS groups. The between subjects factor was group, with three levels (control, left MTS, right MTS) and the within subjects factor was hemisphere, with two levels (left, right). Both factors were assumed to be fixed effects. Separate ANOVA procedures were carried out for hippocampus, ErC and PrC. The ANOVA summary table is shown in Table 3A—C.

As can be seen in Table 3A, for the hippocampus, the main effect of hemisphere is not significant, however the main effect of group is significant. The two-way interaction between hemisphere and group is also significant. This interaction is plotted in Fig. 2A. Examination of the figure shows that in the left hemisphere, patients with left MTS have smaller hippocampal volume than those with right MTS (post hoc *t*-test, $p < 0.001$), while in the right

Table 2 Mean and standard deviation (μ (S.D.)) of hippocampal, entorhinal cortex and perirhinal cortex volumes, shown separately for the control group, left MTS and right MTS groups

Structure	Control ($n = 20$) (mm^3)	Left MTS ($n = 33$) (mm^3)	Right MTS ($n = 28$) (mm^3)
Left hippocampus	3176.89 (445.90)	1837.01 (481.03)	2737.77 (494.27)
Right hippocampus	3234.11 (409.40)	2920.69 (466.29)	1804.68 (434.38)
Left ErC	837.09 (141.39)	693.74 (156.87)	799.45 (210.79)
Right ErC	808.98 (148.84)	708.06 (164.21)	689.37 (196.56)
Left PrC	1444.65 (310.98)	1218.99 (323.04)	1485.25 (398.30)
Right PrC	1408.65 (308.80)	1319.25 (409.41)	1276.05 (406.34)

hemisphere the order of means is reversed ($p < 0.001$). In this, and all subsequent comparisons, post hoc t-tests on interactions are reported with Bonferroni corrections for multiple comparisons. The hippocampal volume of control subjects does not differ between hemispheres, and is larger than in both left MTS ($p < 0.01$) and right MTS ($p < 0.001$) patients.

For the ErC, Table 3B shows the main effect of hemisphere is significant, indicating that ErC volumes vary across hemispheres. The main effect of group is also significant and the two-way interaction between hemisphere and group is significant. The interaction is plotted in Fig. 2B. Examination of Fig. 2B shows that in the left hemisphere, as with hippocampal volume, patients with left MTS have smaller ErC volume compared to controls ($p < 0.01$), while the ErC volume in right MTS patients approaches that of controls. However, both left and right MTS have significantly reduced right hemisphere ErC volume ($p < 0.05$ and <0.01, respectively).

With respect to PrC volumes, Table 3C shows that the main effect of hemisphere is significant, however the main effect of group is not significant. The two-way interaction between hemisphere and group is significant. The interaction is plotted in Fig. 2C. Fig. 2C shows in the left hemisphere, as with hip-

Table 3 ANOVA summary table contrasting hemisphere (left, right) with subject group (control, left MTS, right MTS) for (A) hippocampal (B) entorhinal cortex and (C) perirhinal cortex volume

Source	SS	d.f.	MS	F	p	η^2
Hippocampal						
Within subjects effects						
Hemisphere	186106.16	1	186106.16	2.62	0.109	0.033
Hemisphere × group	30881669.26	2	15440834.63	217.67	0.000	0.848
Error (hemisphere)	5533175.30	78	70938.15			
Between subjects effects						
Group	23469354.51	2	11734677.25	33.31	0.000	0.461
Error	27481924.10	78	352332.36			
Entorhinal cortex						
Within subjects effects						
Hemisphere	65383.55	1	65383.55	10.96	0.001	0.125
Hemisphere × group	116288.07	2	58144.04	9.74	0.000	0.202
Error (hemisphere)	459448.52	77	5966.86			
Between subjects effects						
Group	371707.63	2	185853.81	3.45	0.037	0.082
Error	4151427.59	77	53914.64			
Perirhinal cortex volume						
Within subjects effects						
Hemisphere	89504.69	1	89504.69	4.55	0.036	0.056
Hemisphere × group	711122.25	2	355561.12	18.06	0.000	0.319
Error (hemisphere)	1515743.62	77	19684.98			
Between subjects effects						
Group	715110.92	2	357555.46	1.43	0.246	0.036
Error	19254140.35	77	250053.77			

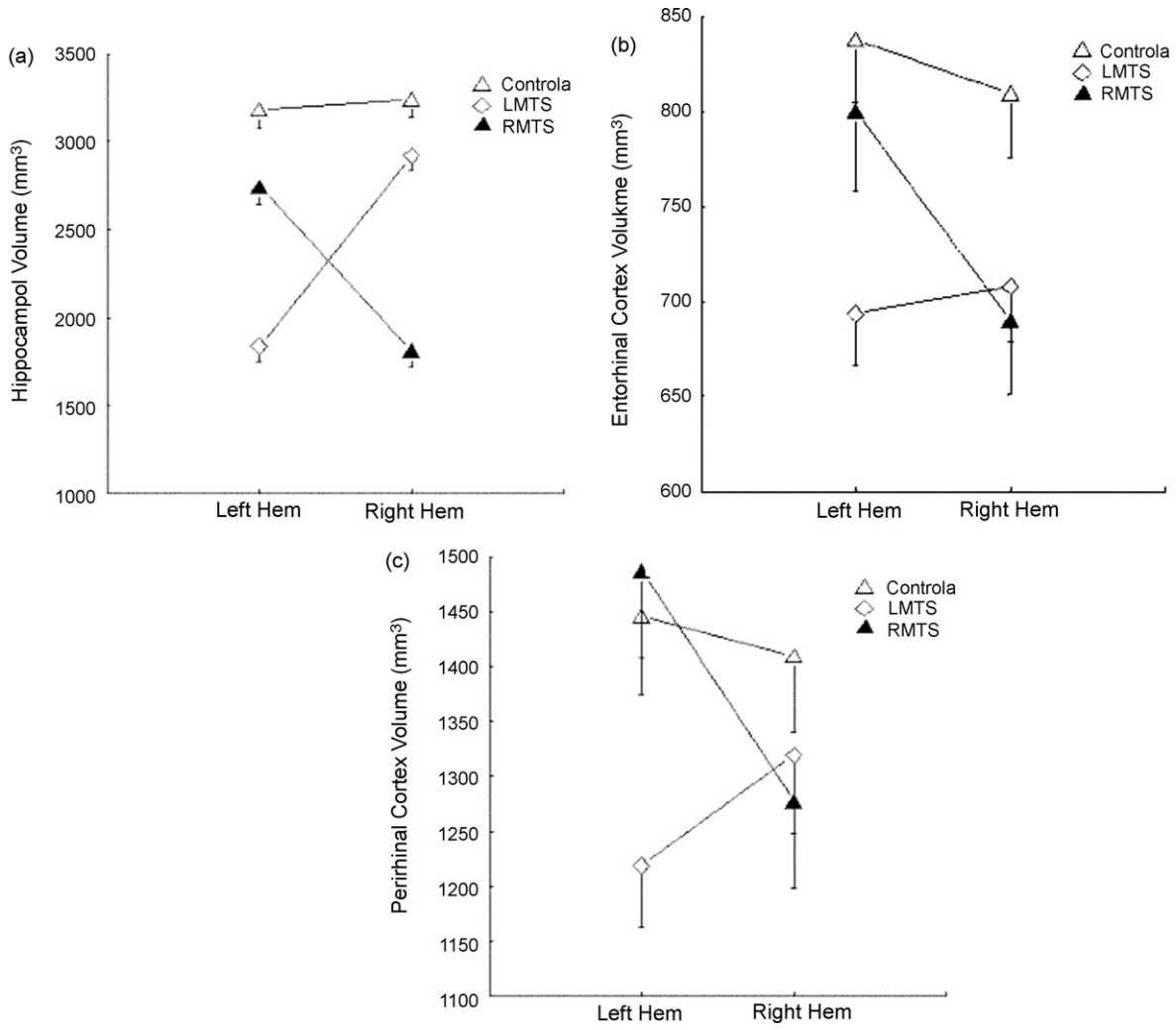

Figure 2 (A) The two-way interactions between hemisphere (left, right) and group (control, left MTS, right MTS), shown separately for mean hippocampal volumes. For this figure and figures B and C (see below), the open triangles represent the control group, the open diamonds represent the left MTS group and the closed triangles represent the right MTS group. (B) The two-way interactions between hemisphere (left, right) and group (control, left MTS, right MTS), shown separately for mean entorhinal cortex volumes. (C) The two-way interactions between hemisphere (left, right) and group (control, left MTS, right MTS), shown separately for mean perirhinal cortex volumes.

pocampal and ErC volume, patients with left MTS have reduced PrC volume relative to controls ($p < 0.05$), while PrC volume in right MTS patients is not significantly different from controls. However, in the right hemisphere, neither disease group shows significantly reduced volumes.

Pearson correlational analysis was used to further examine hippocampal and rhinal cortex volumes in the control group and the left- and right-sided MTS groups. A strong relationship was observed across hemispheres for each structure, in the control groups (range 0.74—0.92) and both left and right MTS groups (range). In the patient group, the pattern of relationships between ErC and PrC differs between right and left MTS groups. In right MTS patients, ErC and PrC were highly correlated within hemispheres. However, in patients with left MTS, only right-handed patients show significant intrahemispheric correlations. In addition, many of the correlations represent large experimental effects.[31] Interestingly, hippocampal volumes do not correlate significantly with ErC or PrC volumes in either the control group or the disease groups (full details of the correlations available on request from the authors).

As depicted in Fig. 2A—C, no differences were found in the control group for left and right hemisphere volumes of the hippocampus ($t_{(19)} = 1.47$, $p = 0.159$, $d = 0.14$), ErC ($t_{(19)} = 1.20$, $p = 0.244$, $d = 0.12$), or PrC ($t_{(19)} = 0.79$, $p = 0.438$, $d = 0.20$). As such, the control group was excluded from any further analysis involving hemispheric comparisons.

Table 4 Mean and standard deviation (μ (S.D.)) of hippocampal, entorhinal cortex and perirhinal cortex volumes for patients with left MTS (n = 33) and those with right MTS (n = 27)

Handedness	Hippocampus		Entorhinal Cortex		Perirhinal Cortex	
	Left hemisphere (mm^3)	Right hemisphere (mm^3)	Left hemisphere (mm^3)	Right hemisphere (mm^3)	Left hemisphere (mm^3)	Right hemisphere (mm^3)
Left hippocampal sclerosis						
Left (n = 9)	2249.80 (548.35)	3046.11 (622.39)	743.49 (138.93)	704.65 (152.32)	1404.09 (403.95)	1599.74 (541.39)
Right (n = 24)	1682.22 (354.49)	2873.66 (399.13)	675.09 (161.84)	709.35 (171.59)	1149.57 (264.58)	1214.07 (298.20)
Right hippocampal sclerosis						
Left (n = 6)	2726.50 (464.55)	1909.67 (393.83)	721.20 (136.15)	574.20 (106.46)	1487.41 (401.66)	1174.14 (375.38)
Right (n = 21)	2716.30 (511.71)	1788.05 (456.52)	821.81 (225.31)	722.27 (205.61)	1484.63 (407.31)	1305.17 (418.82)

Values shown separately for left and right hemisphere and handedness.

Subsequent analyses focused on the relationship between hemispheres in patients with MTS. A five-way ANOVA was performed with three between subjects factors, side of MTS and handedness with two levels (left, right), and gender with two levels (female, male). There were two within subjects factors, hemisphere, with two levels (left, right), and anatomical structure, with three levels (hippocampus, ErC and PrC). Descriptive statistics (means and standard deviations) are shown in Table 4.

Full details of ANOVA analysis are available on request from the authors. Results of analysis show that the main effect of structure is significant, indicating that hippocampal, ErC and PrC structures differ in size [$F(2,104) = 293.71$, $p < 0.001$, $\eta^2 = 0.85$]. However, the main effect of hemisphere is not significant, neither was the main effect of side of MTS, nor handedness, nor gender significant. Examination of the nonsignificant trend of gender on volume ($p = 0.056$) using Bonferroni post-hoc analyses showed that males have significantly larger PrC volumes than females in left [$t_{(31)} = 3.03$, $p = 0.005$] and right [$t_{(25)} = 3.36$, $p = 0.003$] MTS groups. There are no significant gender differences for hippocampal or ErC volume.

The two-way interaction between hemisphere and side of MTS is significant, indicating that volumes differ in each hemisphere as a function of side of MTS [$F(1,52) = 230.94$, $p < 0.001$, $\eta^2 = 0.82$]. This, and subsequent significant interactions will be interpreted as part of the significant higher-order interactions. The two-way interaction between hemisphere and structure is significant, indicating that structures differ in size in left versus right hemispheres [$F(2,104) = 4.82$, $p < 0.05$, $\eta^2 = 0.09$]. The two-way interaction between side of MTS and handedness is also significant, as is the two-way interaction between structure and gender [$F(1,52) = 5.27$, $p < 0.05$, $\eta^2 = 0.09$] and [$F(2,104) = 3.17$, $p < 0.05$, $\eta^2 = 0.06$], respectively. When corrected for violation of the assumption of sphericity, the structure by gender interaction just ceases to be significant ($p = 0.06$), but all other interactions remain significant. None of the other two-way interactions are significant.

The three-way interaction between hemisphere, structure and side of MTS is significant, indicating that the three anatomical structures differed as a function of hemisphere and side of MTS [$F(2,104) = 158.08$, $p < 0.001$, $\eta^2 = 0.75$]. This interaction is apparent in Fig. 2A–C for the disease groups. Examination of the means suggests that in the left hemisphere, patients with left MTS have smaller hippocampal and PrC volume compared to those with right MTS ($p < 0.05$), while in the right hemisphere, patients with right MTS have smaller

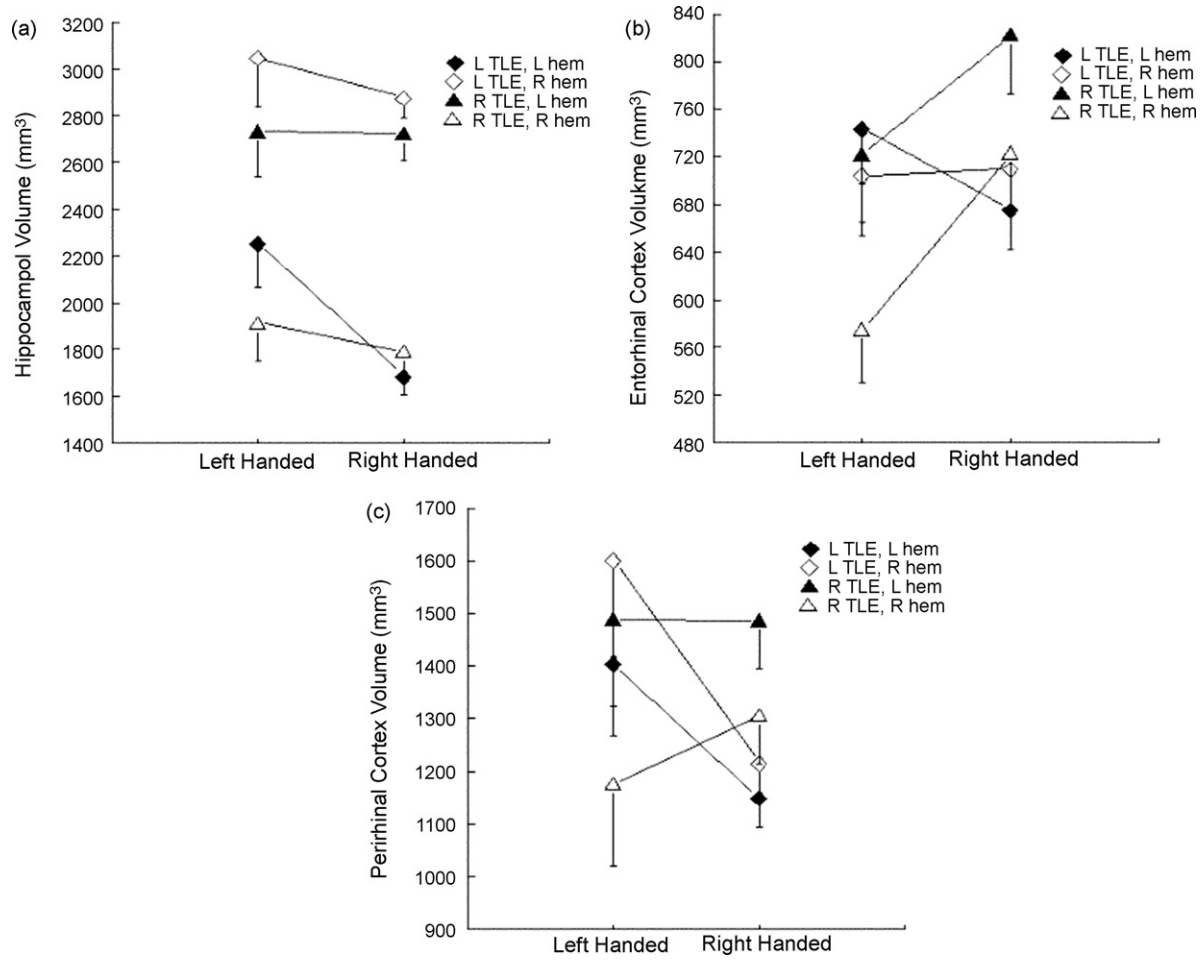

Figure 3 (A) The four-factor interaction between side of MTS (left, right), handedness (left, right), hemisphere (left, right) and structure, shown separately for mean hippocampal volume. For this figure and figures B and C (see below), the closed diamonds represent the left hemisphere in the left TLE group, the open diamonds represent the right hemisphere in the left TLE group, the closed triangles represent the left hemisphere in the right MTS group and the open triangles represent the right hemisphere in the right TLE group. (B) The four-factor interaction between side of MTS (left, right), handedness (left, right), hemisphere (left, right) and structure, shown separately for mean entorhinal cortex volume. (C) The four-factor interaction between side of MTS (left, right), handedness (left, right), hemisphere (left, right) and structure, shown separately for mean perirhinal cortex volume.

hippocampal and ErC volumes than those with left MTS ($p < 0.05$). None of the other three-way interactions are significant.

The four-way interaction between hemisphere, structure, side of MTS and handedness is significant [$F(2,104) = 5.08$, $p < 0.01$, $\eta^2 = 0.09$]. However, none of the other four-way interactions were significant, neither was the five-way interaction between hemisphere, structure, side of MTS, handedness and gender significant. Subsequent analysis will focus on interpretation of the four-way interaction which is plotted in Fig. 3.

Post hoc contrasts showed that for the hippocampus (Fig. 3A), when disease affects the left hemisphere, ipsilateral volume is larger in left-handed patients than right handers ($p < 0.001$). However, when disease is in the right hemisphere, there is no effect of handedness on ipsilateral volume. So handedness has a significant effect on ipsilateral hippocampal volume in patients with left sided disease only. In addition, volumes contralateral to the side of MTS are larger, irrespective of side of MTS or handedness ($p < 0.001$).

The ErC shows the reverse pattern (Fig. 3B). For left MTS patients there is no obvious effect of hemisphere or handedness on ErC volume. In the right MTS, however, right hemisphere volumes are larger in right handers than left handers ($p < 0.05$). In addition, in the right MTS group, the contralateral volumes (i.e. left hemisphere volumes) are larger than ipsilateral volumes ($p < 0.01$), regardless of handedness.

The PrC shows a similar pattern to the hippocampus, whereby patients with right MTS show larger

contralateral volumes ($p < 0.01$) and there is no obvious effect of handedness on this group (Fig. 3C). For left MTS patients, there is a dramatic effect of handedness on volume, whereby volumes are larger in left handers, regardless of hemisphere ($p < 0.05$). In addition, in left MTS patients, there is a trend for contralateral volumes to be larger, but not significantly so.

In light of the handedness effects revealed in the patient group, this factor was re-examined in the control group. There was no effect of handedness on hippocampal, ErC or PrC volume in either hemisphere. However, it should be noted, the sample size of left handers in this group was small ($n = 2$).

In summary, across all structures there is an effect of dominance, which differs between disease groups. Dominance acts to reduce hippocampal and PrC volume in left MTS patients, and ErC volume in right MTS patients.

The effect of age on volume was examined for each structure, using correlational analysis. Age has no significant effect on volume of any structure for controls [hippocampus ($r = -0.30$, $p = 0.201$); ErC ($r = 0.12$, $p = 0.617$); PrC ($r = 0.01$, $p = 0.954$)], left MTS [hippocampus ($r = -0.20$, $p = 0.268$); ErC ($r = 0.18$, $p = 0.306$); PrC ($r = -0.13$, $0 = 0.469$)], or right MTS [hippocampus ($r = -0.32$, $p = 0.092$); ErC ($r = -0.09$, $p = 0.656$); PrC ($r = 0.02$, $p = 0.932$)] groups.

Discussion

Rhinal cortex volumes in patients with MTS compared to normal controls

In this quantitative MRI study, we have demonstrated reliable *in vivo* evidence of morphometric changes in both ErC and PrC in a substantial sample of patients with unilateral MTS, compared to normal controls. For the hippocampus, the characteristic patterns of MTS pathology were reflected in reduced volumes ipsilateral to the side of disease, with contralateral volumes not significantly different from controls. Rhinal cortex volumes were reduced as a function of laterality of disease. In left MTS patients, PrC volume was reduced, but only in the left hemisphere, while ErC volume was bilaterally reduced. In right MTS patients, right hemisphere ErC volume was reduced, while PrC volumes did not differ from controls in either hemisphere.

The findings accord with previous studies which have demonstrated ipsilateral ErC cortex reductions in patients with MTS.[18–20] Unlike the former studies, patients in the present study were a homogenous group of patients, with histopathologically confirmed unilateral MTS in all but two cases. In addition, the present study examined a significantly larger number of patients. The present data demonstrate that the unilateral pattern of ErC volume reductions is limited to right MTS patients. Left MTS patients show bilateral reduction in ErC volumes.

One previous study examined PrC in TLE patients and documented PrC volume reduction in two of six patients assessed.[23] They found volume changes to be more pronounced in the ErC than PrC, however the distributional properties of volume changes were not addressed in this study. The present findings shed further light on the patterns of PrC volume changes, demonstrating the reverse pattern to ErC volumes, whereby left MTS show ipsilateral PrC reduction, and right MTS patients showed no volume reduction in either hemisphere. In other words, rhinal cortex volume changes in patients with MTS may be influenced by laterality of disease.

Of note, the rhinal cortex structures are small and inevitably, as with any small sample, the results of this study require replication to establish the generalisability of the findings.

Relationships amongst hippocampal, entorhinal cortex and perirhinal cortex volumes

The study also permitted a within subject analysis of rhinal and hippocampal volumes. The findings suggest the existence of a strong relationship across hemispheres for hippocampus, ErC and PrC volumes in patients with MTS, and neurologically normal controls. In the patient group, the pattern of relationships between ErC and PrC again varied with laterality of disease. In right MTS patients, ErC and PrC were highly correlated within hemispheres, while in patients with left MTS, the significant intrahemispheric correlations were only present in right handers. It appears that relationships within the rhinal cortex, at least in left MTS patients, vary with handedness. The relationship between ErC and PrC may be independent from hippocampal disease status. The correlations suggest that ErC and PrC are closely related structures, which function independently of the hippocampus. These findings will be addressed in the discussion to follow.

The present findings suggest that in addition to ErC, the PrC cannot be assumed to be normal in patients with MTS. In accordance with previous studies, some inter-individual variability was observed in ErC and to a larger extent PrC volumes measurements.[26,29] A direct comparison of the present data with earlier findings from other groups is difficult, however, due to variability in data acquisition techniques and analysis software, as well as

variability in anatomical protocols.[28] The volumetric imaging methods used in our centre to measure hippocampal and amygdala volumes are reliable,[16] and have produced results comparable to the bulk of volumetric studies from other centres.[32] The ErC and PrC volume protocols used in this study were also found to be reliable.

Factors affecting rhinal cortex and hippocampal volume—lateralised findings

So why should the pathogenesis of MTS in rhinal cortex structures be reflected differentially depending on the laterality of the disease? A lateralised distribution of brain disease has been reported in other neurodevelopmental abnormalities, such as external birth defects.[33] For example, Fantel and colleagues reported that the right side of the rat embryo was more susceptible to hypoxic damage than the left.[34] The reasons for the axially asymmetric defects are not clear, but may relate to hemispheric differences in the rate of mitochondrial maturity,[35] or subtle hemispheric differences in the vascular supply.[36] Perhaps the right and left hemispheres are also differentially affected by MTS disease, as a consequence of variations in cellular structure or vascularity.

The interaction between handedness, laterality of disease and hemisphere in the hippocampus and rhinal cortex was a novel finding, which shed further light on the laterality findings. The influence of handedness differentially related to the laterality of MTS. For the hippocampus, handedness had an effect exclusively on the left MTS patients, left hemisphere volumes being larger in left handers than in right handers. No handedness effect was observed in patients with right MTS. A handedness effect on PrC was seen exclusively in left MTS, whereby left handers showed larger volumes than right handers. In the case of left handers, the differences were evident in both left and right hemispheres. In contrast, the handedness effect on ErC was only evident in right MTS patients, where right handers had larger bilateral volumes than left handers. An association between handedness and brain volume changes has not previously been documented in this patient group, to our knowledge.

The mechanism by which cerebral dominance could modulate the pathogenesis of mesial temporal sclerosis is a matter of speculation as we are not aware of any relevant published research in this area. Assuming our results reflect representative sampling, then understanding the pattern of results depends upon determining whether pathogenesis predates the development of cerebral dominance in ontogenesis, or visa versa. Should subtle hemispheric asymmetries occur at a cellular level or in vascular irregularities, however, it would date the genesis of MTS to very early development, perhaps early within the first trimester. The cause and pathogenesis of MTS has been a source of controversy over the last century, and remains unresolved.[15,37] One etiological theory argues for the occurrence of a cerebral insult early in life, perhaps perinatally, which marks the beginning of the disease.[15,38] Intracerebral infections or perinatal trauma have also been proposed as possible explanations, but no direct evidence has been found to support these theories. The present data may suggest that research be directed to very early perinatal irregularities as a causal hypothesis in MTS.

Functional distinctions within the limbic system?

The relationships between hippocampal and rhinal cortex structures in regulating human memory have been difficult to define, due to the difficulties in characterising these structures *in vivo*.[27,39]. In the experimental literature, recent reviews have highlighted the role of the ErC, and particularly the PrC, as key structures in visual object recognition.[40–42] The contribution of the hippocampus to recognition memory, however, has been more controversial.[41] Differences in experimental lesion methods have further complicated interpretation of results. While some studies have reported no recognition memory deficit following hippocampal lesions,[43] others have reported mild impairments,[44] or even severe deficits.[45] It has been suggested that the role of the rhinal cortex and hippocampus may be dissociable, so that while rhinal cortex is important for recognition memory, the hippocampus may be more relevant to spatial memory, or memory for places.[1,46]

There are two separate findings from the present study that may contribute some indirect evidence to these functional controversies. The patterns of volume changes in the hippocampus and PrC were different to that seen in the ErC. In addition, the correlational data suggested that rhinal cortex volumes were highly related but independent of hippocampal volumes. Baxter and Murray[41] reviewed studies of analogous structures in monkeys performing recognition memory tasks. They found that while PrC lesions produced a convincing recognition memory impairment, damage to hippocampal structures was inversely related to the magnitude of the deficits. That is, larger hippocampal lesions gave rise to milder memory impairments. One explanation offered for the findings was that the two structures contribute to the solution of memory tasks through different cognitive strategies. So while PrC

and hippocampus make similar contributions to memory, their functional capacity may be impaired in different ways by pathology. A functional distinction would be consistent with the neuropathological data suggesting that the hippocampus and PrC are not directly connected.[6]

One plausible extension could be made from the findings of Baxter and Murray[41] to the present data. The patterns of volume changes observed in our study would be consistent with a distinction between the role of PrC and hippocampus in memory function, versus the ErC. In contrast, the correlational data in the present study suggest that the rhinal cortex and hippocampus may be independent in terms of volumes. This would support the hypothesis of Baxter and Murray that the role of the rhinal structures in regulating memory may be functionally separate from the role of the hippocampus, although in human disease, such as MTS, the pattern of pathology includes both rhinal cortex and hippocampus. Quantitative MRI may permit the exploration of the nature of the memory disturbance in humans, including the possibility that an inverse relationship exists between volume and memory performance.

Drawing functional hypotheses from structural relationships is necessarily speculative. The present study has contributed novel information about the patterns of rhinal pathology in patients with MTS. Combining the quantitative imaging protocols with detailed memory and cognitive examination is necessary to understand the functional significance of the hippocampal and rhinal cortex changes, and their association with handedness and laterality of MTS in these patients.

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