Relative localizing value of amygdalo-hippocampal MR biometry in temporal lobe epilepsy

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Abstract

Objectives: The aims of the study were (i) to examine the localizing value of three MRI quantitative modalities (qMRI) currently used for the analysis of the hippocampus and amygdala in the context of pre-surgical screening and (ii) to propose a step-by-step protocol based on the sensitivity and performance of the different MR techniques.

Methods: Ninety-two adults with chronic mesial temporal lobe epilepsy (TLE) of which 28 underwent amygdalo-hippocampal resection, and 34 age-matched controls were included in the study. High-resolution qMRI was performed at 1.5 T, including a tilted T1-weighted 3D-dataset for volumetry and four-echoes T2 relaxometry (both for hippocampus and amygdala quantifications) and multi-voxel spectroscopy [NAA/(Cho + Cre)] (exclusively in the hippocampus). Individual qMRI data were compared with electroencephalography regarding the localization of the epileptogenic area, with the neuropathological data and with postoperative outcome. MRI pathology was defined based on 99% confidence ellipses. Ten controls were used to assess the quantitative MRI intra- and inter-observer variability for all variables.

Results: Volumetric measurements revealed unilateral damage in 77% of the patients, T2-relaxometry in 64% and spectroscopy in 53%. Additional measurements of the amygdalae (T2-relaxometry) allowed us to localize pathology that coexists with that of the hippocampus in 34%, and isolated unilateral amygdala damage in 8% of patients. Volumetry and T2-relaxometry (not spectroscopy) were associated with postoperative outcome, but accurate predictive models were computed based on hippocampal measures only. At least at 1-year follow-up, volumetry predicts outcome correctly in 100% of the cases, whilst T2-relaxometry classified 96.4% (27/28) of these patients. All operated patients had hippocampal sclerosis.

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Conclusions

Hippocampal structural damage is equivocally depicted by spectroscopy. For diagnostic and pre-operative evaluation, hippocampal volumetry and T2-relaxometry provide maximal accuracy. Amygdala quantifications are irrelevant in the pre-operative evaluation but may be useful for diagnostic purposes. Of the three qMRI modalities tested, T2-relaxometry provided the best balance between diagnosis accuracy and time-efficiency to lateralize a sclerotic lesion on the majority of the patients. Cases that remain undecided after T2-relaxometry may benefit from additional measurements based on hippocampal volumetry.

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1. Introduction

Magnetic resonance imaging (MRI) provides an objective means for studying the individual temporal lobe structures in vivo and contributes unambiguously to the identification of patients with the mesial temporal epilepsy (TLE) syndrome (ILAE, 2004) that are refractory to pharmacotherapy and have a surgical indication.

The standard surgical treatment of patients with manifest TLE is anterior temporal lobectomy, which includes the removal of the hippocampus and the amygdala (Yasargil et al., 1985; Wiebe et al., 2001). This effective procedure leads to improvement in up to 85% of properly selected patients. To avoid undesired effects and to optimize the results of the surgical intervention, it is necessary to identify and lateralize the seizure focus with a high degree of accuracy.

Unilateral hippocampal sclerosis (HS) is the most common pathological diagnosis in TLE, and up to 65% of cases of TLE may be attributed to lesions arising exclusively in the hippocampus (Babb, 1992; Jutila et al., 2002). Histopathological studies have reported a concomitant pattern of tissue damage, that is, selective neuronal loss and gliosis extending to the amygdala [10–60% of the cases with a damaged hippocampus (Cavanagh and Meyer, 1956; Margerison and Corsellis, 1966; Yilmazer-Hanke et al., 2000)] and the para-hippocampal gyri [21–68% of the cases (Cavanagh and Meyer, 1956; Yilmazer-Hanke et al., 2000)]. It has also been reported that isolated amygdala damage can be present in up to 10% of the patients referred for surgical treatment (Swartz et al., 1992; Miller et al., 1994; Lee et al., 1998). Such widespread involvement of mesial temporal lobe structures is usually denoted by the term “mesial temporal sclerosis” (MTS).

Quantitative imaging evaluations (qMRI) are relatively unbiased when compared with qualitative observations and provide a robust means to determine the severity and extent of tissue damage in the mesial temporal lobe. Moreover, several qMRI techniques provide useful prognostic information regarding surgical outcome of those patients who are refractory to pharmacotherapy [volume: Jack et al. (1992), Cascino et al. (1995) and Cendes et al. (1997); T2-relaxometry: Kuzniecky et al. (1997); spectroscopy: Kuzniecky et al. (1999) and Antel et al. (2002); diffusion (ADC-mapping): Gonçalves Pereira et al. (2006)].

However, current qMRI analyses have the disadvantage of being time-consuming when compared with qualitative observations. Additionally, volumetry studies depend critically on the observer’s skills while T2-relaxometry and spectroscopy need dedicated software. Thus, whenever a qMRI protocol is installed for TLE pre-surgical screening a balance must be thought off which considers the necessary investment in personnel training and software acquisitions.

Comparative qMRI studies have been essentially conducted in the hippocampus, namely hippocampal volumetry and spectroscopy (Cendes et al., 1997; Kuzniecky et al., 1998); hippocampal volumetry and T2-relaxometry (Van Paesschen et al., 1995; von Oertzen et al., 2002); hippocampal volumetry, spectroscopy and diffusion-weighted imaging (Duzel et al., 2004) and also in the amygdala [volumetry and T2-relaxometry (Kalviainen et al., 1997)]. Nevertheless, no study has so far investigated the relationship between hippocampal qMRI variables [volumetry (HCVOL), T2-relaxometry (HCT2) and multi-voxel spectroscopy (HCSI)] jointly with amygdala volumes (AMYVOL) and T2 maps (AMYT2).
The goal of this study was to assess the relative value of volumetry, T2 relaxometry and spectroscopy as such and in combinations, in the evaluation of the mesial temporal integrity (hippocampus and amygdala) given the context of pre-surgical screening. This is a relevant issue since no single technique may be sufficiently sensitive to detect the whole spectrum of hippocampal and amygdala pathology. An essential question is whether these techniques provide complementary or redundant information about the status of the mesiotemporal structures. This information may help us to optimize the scanning and post-processing procedure so that a multimodal MR protocol may be established with improved sensitivity in the shortest possible time.

Parts of this work have been published in abstract form (Gonçalves Pereira et al., 2002a,b).

2. Patients and methods

2.1. Control subjects

The control group included 34 subjects (17 women) with a mean age [1 standard deviation (S.D.)] of 33.7, 9.7 (range: 19–52) years. All the volunteers were interviewed to exclude those with neurologic diseases and were submitted to detailed neurological examination and a “mini-mental state” test (Folstein et al., 1975) [obtained score and range was: 28.6, 1.3 (26–30)].

2.2. Patients

Ninety-two patients (51 women) with a mean age of 32.9, 9.8 (17–63) years were enrolled in the study. Classification of the patients syndromes was based on the guidelines proposed by the International League Against Epilepsy (ILAE, 1989). All had medically refractory TLE and underwent comprehensive presurgical evaluations, which included a detailed clinical history, interictal EEG, prolonged video-telemetry monitoring, MRI studies, neuropsychological assessment and intracarotid sodium amobarbital tests (in selected cases). Only patients with clinical and EEG evidence of TLE and without any non-neoplastic, vascular, developmental or non-hippocampal volume loss demonstrated at MR imaging were included.

2.3. MR Image acquisition

All MR studies were obtained on the same 1.5 T scanner (CV/i-NV/1 equipped with high-capacity gradient (amplitude: 40 mT/m, slew rate: 200 (T/m)/s), General Electric Medical Systems, Milwaukee, WI, USA), over a period of approximately two years (May 2001–August 2003). Every subject underwent a single MRI session. During the scanning period (approximately 70 min) all participants were comfortably placed and their heads were fixated within the headcoil with customized cushions. Special attention was paid to the symmetric positioning of the subjects head. Imaging processing was performed using the manufacturer’s software, GE Advantage Windows 3.1 (including research modules), running on Sun workstations (SPARC 4.1, Sun Microsystems, Mountain View, CA, USA).

Presurgical imaging consisted of standard qualitative sequences (multiplanar high-resolution T1- and T2-weighted, proton density, inversion-recovery and fluid-attenuation inversion recovery scans). For this study, patients and controls were further evaluated with a specific MR protocol in order to obtain quantitative data, part of which was described elsewhere (Gonçalves Pereira et al., 2006).

All sequences were obtained using a tilted orientation to the temporal lobes, orthogonal to the axis of the hippocampal body (Gonçalves Pereira et al., 2005). Imaging for hippocampal and amygdala volume and relaxometry (Fig. 1) was acquired on the coronal plane, parallel to the posterior comissure-obex line (PC-OB) (Tamraz and Comair, 2000). Two-dimensional (2D) multi-voxel hippocampal spectroscopy was acquired on the axial plane perpendicular to PC-OB (Fig. 1).

A sagittal spin-echo T1-weighted with TR = 600 ms, TE = 15 ms, NEX = 2, FOV = 24 cm, section thickness = 5 mm, gap = 1 mm, acquisition matrix = 512 × 192 pixels was acquired as a reference series for the following sequences (Table 1).

2.3.1. Volumetry

For volumetric studies, we used a T1 three-dimensional spoiled gradient-echo (3D SPGR) protocol with TR = 20–30 ms, TE = Min. Full, NEX = 2, FOV = 24 cm, section thickness = 1.5 mm, gap = 0 mm, acquisition matrix = 512 × 224 pixels, producing a
Fig. 1. Multimodal quantitative MRI as performed in a control subject. (A–C) Hippocampus. (D and E) Amygdala. Coronal T1-weighted MR images demonstrate the outlining of the cross-sectional areas of the hippocampus (A) and amygdala (D) as performed on the workstation (1 marks the first ROI to be drawn, in the structure of the right side). Highlights of coronal T2-weighted relaxometry images, demonstrate the positioning of the ROIs over the hippocampal body (B) and amygdala (E), for direct calculation of the relaxometry values. Axial T2-weighted localizer image for multi-voxel spectroscopy (C), demonstrate the positioning of the volumes of interest over the hippocampus. Individual NAA, Cho and Cre values are obtained for each voxel (see text for other details).

series of 118–126 slices of the entire brain. Right and left hippocampus and amygdala volumes were measured following the general guidelines of Watson et al. (1992) on images magnified four-fold, with some modifications. For each subject, the posterior and anterior limits of each hippocampus were identified on the right and left para-sagittal sections, where the corresponding in-plane coronal images (with the reference image number) could be displayed. The posterior limit of the amygdala was identified using the same methodology. However, with current MRI, the anterior limit of the amygdala is difficult to identify by means of its different contrast and, thus we had to refer to external anatomical references [for a review see Brierley et al. (2002)]. We followed the borders described by Cendes et al. (1993) and Soininen et al. (1994) with modifications (for the anterior limit, we choose the level where the lateral sulcus closes to form the endorhinal sulcus and, simultaneously, the optic chiasm appears continuous).

Volumes were calculated by measuring the hippocampal and amygdala areas within each slice using the manufacturer’s interactive mouse-driven software (Fig. 1) and multiplying it by the thickness of the section; the resulting volumes were summed to obtain a total volume [Cavalieri slices method (Gundersen and Jensen, 1987; Roberts et al., 2000)].

Finally, to correct the absolute volumes for the individual variance in head size a ratio was used which allows obtaining ‘normalized volumes’. We adopted the procedure of Cendes et al. (1994), with the modifications of Kalviainen et al. (1997). This procedure allows obtaining comparable volumes of the structures of interest in every subject, taking into account the differences in volume of the total brain. As subjects consecutively differ in morphometric parameters, including cranial size, overestimations of volumes (and thus misdiagnosis) will occur if two subjects with a proportionally similar hippocampal or amygdala size are compared without correction, when one of the subjects has a larger brain volume.

2.3.2. T2-relaxometry

The method used for T2-relaxometry was similar to that described by Jackson et al. (1993), with some modifications due to time constraints (Pires et al., 1998). We used a 4-echoes protocol (instead of 16) with TR = 3000 ms, TE= 45–90–135–180 ms, NEX=2, FOV = 24 cm, section thickness = 4 mm, gap = 1 mm, acquisition matrix = 256 × 192 pixels, producing a series of 15 oblique slices over the whole temporal lobe. To assure reproducibility, the first section was placed at the posterior end of the corpus callosum, in the mid-sagittal plane.
Table 1

<table>
<thead>
<tr>
<th>MRI method</th>
<th>Summary of technical description</th>
<th>Scanning time (min)</th>
<th>Processing time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volumetry</td>
<td>T1 3D SPGR with continuous 1.5 mm slices (coronal)</td>
<td>12</td>
<td>50–55</td>
</tr>
<tr>
<td>T2-relaxometry</td>
<td>Four-echoes with interleaved 4 mm slices (coronal)</td>
<td>15</td>
<td>10–12</td>
</tr>
<tr>
<td>Multi-voxel spectroscopy</td>
<td>2D-chemical-shift with PRESS and TE = 144 ms (axial)</td>
<td>10</td>
<td>5–8</td>
</tr>
</tbody>
</table>

See text for details. Processing time includes the quantification of hippocampal and amygdala volumes and T2-relaxometry. Multi-voxel spectroscopy was performed only in the hippocampus. PRESS: point-resolved spectroscopy. Time in minutes.

For T2-relaxometry, the data from successive echoes were fitted, pixel by pixel, to a mono-exponential decay curve. Regions of interest (ROI) of arbitrary shape (but constant voxel number) were drawn over the hippocampi (20 mm²) and amygdala (40 mm²), avoiding borders where partial volume effects could alter the mean T2 value for the ROI (Fig. 1). For each subject, the HCT2 results were obtained by averaging ROI’s values from three central slices taken at the level of the body of the hippocampus. AMYT2 were calculated by averaging ROI’s values from two anterior slices, which included the amygdala.

2.3.3. Hippocampal spectroscopy

For HCSI, a T2 fast spin-echo (TR = 4800 ms, TE = 136 ms, NEX = 2, FOV = 24 cm × 18 cm, section thickness = 3 mm, gap = 1 mm, matrix = 256 × 224 pixels) localizer was acquired at an oblique transverse orientation, parallel to the long axis of the hippocampus (perpendicular to PC-OB) to reduce partial volume effects due to surrounding cerebrospinal fluid. A 2D proton magnetic resonance spectroscopy imaging sequence with point-resolved spectroscopic (PRESS) multi-volume selection (TR = 1000 ms, TE = 144 ms, NEX = 1, FOV = 24 cm × 24 cm, 18 × 18 encoding steps, PROBE-S1) was then acquired on the T2 image where the hippocampi were fully visible (Ng et al., 2000; Bernasconi et al., 2002). Shimming (GE Grad-Shim) and water suppression were performed automatically. To exclude the large lipid resonances from the extracranial fat, the length and width of the acquired volume were adjusted for each individual case, depending on head size, whereas section thickness was set to 10 mm in all cases. The volume of interest was placed behind the clivus and oriented in a similar position for all examinations to cover the entire extend of both hippocampi (Fig. 1). Each voxel had the following nominal size: 7.5 mm × 7.5 mm × 9.9 mm (volume: 557 mm³).

The acquired chemical-shift imaging raw data were post-processed using the standard spectroscopy software (GE FuncTool). To obtain HCSI, two to three voxels were placed along the longitudinal axis of each hippocampal structure, avoiding areas of low signal to noise ratio (Fig. 1). In every subject, technically good-quality spectra were obtained using this standard voxel placement. Mean metabolic concentration was obtained on each hippocampus for N-acetyl-aspartate (NAA), choline (Cho) and creatine/phosphocreatine (Cre) from signal intensities at 2.0 ppm (NAA), 3.0 ppm (Cre) and 3.2 ppm (Cho). The NAA/(Cho + Cre) ratio was then calculated for each hippocampus, since this is a reliable measure for optimal lateralization (Ende et al., 1997). All spectra were inspected thoroughly, and those who were artifactually broadened [i.e., with a full width at half maximum of >12 Hz (water resonance)] or if Cho and Cre peaks were not resolved, were discarded. In a small number of subjects (n = 12), we had to repeat the PROBE-S1 acquisition due to head motion.

For all the MRI variables, an asymmetry index (AI) was used to ascertain the degree of asymmetry (in percentage) between the values obtained in the right (R) and left (L) structures, according with Bernasconi et al. (1999) as follows:

\[ AI(\%) = 100 \times \frac{R - L}{(R + L)/2} \]

2.4. EEG data

All patients were submitted to prolonged in-patient video-EEG monitoring, while anti-epileptic medication was reduced. Scalp electrodes were placed according to the International 10–20 System (Jasper, 1958) with three pairs of additional electrodes over the inferior temporal areas. The EEG signals were digitalized.
and analysed by experts with reformatting facilities and synchronized video.

Lateralization was decided based on semiology (video) and on the electrophysiological ictal activity. In the very few patients (n = 11) without seizures during video-EEG, the presence of a large majority of interictal activity localized to one hemisphere in the fronto-temporal areas was accepted as indicating lateralization (>80%, that is a ratio greater than 4:1 of interictal spikes) (Cendes et al., 2000). Patients whose EEG did not fit these criteria were not enrolled in the study.

2.5. Post-surgical outcome

To date, 28 patients (13 women), with a mean age of 31.8, 9.6 (18–57) years underwent selective amygdalohippocampectomy accordingly to the procedure of Yasargil et al. (1985) and had duration of the follow-up period longer than 1 year. The mean postoperative follow-up was 28 months (range: 13–39). In this group of patients, no reoperations were performed. Patients were assessed by epileptologists on a regular basis (months 3, 6, and 12) and yearly afterwards.

The surgical outcome with respect to seizure control was assessed by means of Engel’s classification (Engel et al., 1993). Patients were classified as having a good outcome (Engel’s class IA) or a less satisfactory outcome (classes IB to III). Such data was related to the lateralizing ability of qMRI measures, its degree of asymmetry and the co-occurrence of hippocampal and amygdala abnormalities. Post-surgical quality of life, memory and disability were not assessed in this study.

2.6. Pathological examination

The neuropathological data collected for this study was based on reports carried out by experienced pathologists.

The group of patients that underwent surgery (n = 28) was from the “Egas Moniz” Hospital (n = 23), “Santa Maria” Hospital (n = 4) and “Hospitais Universitários de Coimbra” (n = 3) Epilepsy Surgery Programmes.

Generally, the surgical procedure was similar across centres. Patients underwent anterior temporal lobectomy with resection of the rostral and mid hippocampus by means of microneurosurgery. The hippocampal head and anterior body were resected en bloc, and the posterior body was then removed by subpial aspiration. This technique removes about 1.5–3.0 cm of the total anterior–posterior length of the hippocampus, depending on which temporal lobe is being resected (1.5 cm for the dominant temporal lobe).

Fresh surgical tissue were fixed using a formaldehyde solution (20%) and later processed into paraffin sections stained with a standard hematoxylin and eosin (H&E) technique for staining neuronal cell bodies. One centre also used the glial fibrillary acidic protein to assess reactive glia.

In all surgical cases, an estimated amount of at least >60% of the resected hippocampus was qualitatively examined, without neuronal counting. Additional mesial temporal tissue was not consistently evaluated in all cases, although parts of the medial amygdala and portions of the subiculum and paras hippocampus were available in a group of patients.

In order to standardize the neuropathological data, our analysis concentrated on the hippocampus and on the observations of the H&E staining, according to established criteria used to diagnose HS (Lantos et al., 2002). As a result, HS was classified into “severe”, “moderate” or “end-folium” depending on the severity and extent of neuronal cell loss. “Severe HS” included major cell loss in sectors CA1, CA3 and dentate hilus, with relative sparing of CA2 and the granular cell layer. “Moderate HS” was diagnosed whenever mild neuronal loss was present in the CA3 and dentate hilus, while CA1 was severely affected. “End-folium HS” consisted of extensive dentate hilar cell loss without a similarly severe loss of dentate granule cells or hippocampal pyramidal neurons.

2.7. Statistical analysis

Group differences for age were assessed using one-way analysis of variance (ANOVA).

Statistics were performed with SPSS Statistical Software Package 11 for Macintosh OS X (SPSS, Inc., Chicago, IL, USA) and STATISTICA for Windows 98 (StatSoft, Inc., Tulsa, OK).

To verify the repeatability of the imaging measurements, the same observer (PMSGP) performed the quantifications twice for all the variables in 10 controls independently with a 6-week interval. To check the inter-observer variability, a second observer (AL)
quantified also the hippocampal volumes. Intra (and inter) observer variability was tested by analysing the limits of agreement between the first and second measurements and the correlations between measurements, according to Bland and Altman (1986), at \( p < 0.05 \). The intra-observer variability was expressed as the means of the coefficients of variation of each control value; it was 3.6% (HCVOL), 4.8% (AMYVOL), 3.1% (HT2), 2.4% (AMYT2), and 0.2% (HCSI). The inter-observer variability expressed in the same way for HCVOL was 4.3%. For all the variables, the mean difference between the two measurements was near zero and the limits of agreement were below 2 standard deviations of the mean of controls.

In order to compare the means of the variables between groups (control versus right TLE; control versus left TLE; control versus non-lateralized), we tested the equality of variances (Levene’s test) and used Student’s \( t \)-test. Tests with respect to differences between the right and left hippocampus and amygdala values were assessed with paired \( t \)-test.

To verify the normal distribution of the values obtained by quantitative MRI, we used the Kolmogorov–Smirnov (Kolmogorov, 1933) and Lilliefors (Lilliefors, 1967) analysis. As the normal distribution was not rejected, we determined confidence ellipses for all the control values, with 99% of confidence.

Confidence ellipses describing values for both right and left hippocampus and amygdala were determined for controls applying the Hotelling’s method (Hotelling, 1936), using STATISTICA software (StatSoft, Inc., Tulsa, OK). The ellipse depends on the correlation coefficient between right and left variables and on data dispersion. The following general equation, which includes the means, the variances and the covariance of both right and left variables of controls, holds:

\[
n(\bar{x} - \mu)^T S^{-1} (\bar{x} - \mu) \leq \frac{(n - 1)p}{(n - p)} F_{p,n-p}(\alpha)
\]

where \( n \) is the number of controls (our case: 34); \( [\bar{x} - \mu] \) the means matrix of the controls; \( [\bar{x} - \mu]^T \) the transpose of a means matrix; \( S^{-1} \) the inverse of the covariance matrix; \( p \) the number of variables (our case: 2, right and left).

In order to assess the level of interdependence between the MR variables, we correlated every hippocampal and amygdala independent values. We also verified whether the magnitudes of the amygdala and hippocampal damage were associated. For this analysis, we tested the level of correlation between volumes, T2 and HCSI values. Pearson’s coefficients (two-tailed) were used to determine the significance levels.

Fisher’s exact test was used to evaluate the probability of association between the qMRI measures, neuropathological data and surgical outcome. Whether such associations were demonstrated, the positive predictive values, odds ratios and Spearman’s correlation’s coefficient were calculated. Additionally, logistic regression models were fitted relating outcome (dependent variable) to the side of surgery and qMRI values on the operated side. One-way analysis of variance (ANOVA) and Spearman’s correlation’s coefficients were used to verify whether there were meaningful differences in qMRI data according to the pathological examination of the hippocampus.

Finally, to compare the discriminative power of the MR techniques for the detection of deviations from normality, that may indicate pathology, and to be able to propose a decision tree for the detection of imaging abnormalities, we analysed both the lateralizing ability of each modality and the corresponding acquisition and operator times.

2.8. Definition of MRI pathology

All the values that fell outside the 99% confidence ellipse for the normal values were considered pathological (Fig. 2). This classification displays on a simple graphical representation all the deviations from normality, which includes patients that have either qMRI values smaller/larger than 3 S.D. from the mean of controls on right and left structures, and those patients whose qMRI values may appear within control range but show an asymmetry index that is larger than 3 S.D. of the mean of controls.

Laterality was defined whenever either of the above conditions was verified and in agreement with the electroencephalographic and clinical data.

3. Results

3.1. Patients groups

Based on neurophysiological evaluation, patients were separated in three groups according to the
A representation of the general confidence ellipse for quantitative MRI calculated with 99% confidence over normal control values. With a simple graphic illustration, every case localized outside the ellipse is immediately categorized as “asymmetrical”, “unilateral” or “bilateral”. Such method can be used to plot every data obtained from paired structures with a normal distribution within the brain.

µ is the mean value, σ is the standard deviation, µ ± 3σ is the mean ± 3 standard deviations.

main seizure electrographic lateralization: right TLE (n = 45), left TLE (n = 39), non-lateralized TLE (n = 8).

The groups did not differ statistically for demographic or illness-related variables.

The patients groups and normal controls did not differ significantly in age [ANOVA; F(3, 122) = 0.31, p = 0.82] or sex distribution [χ²; χ²(1) = 2.1, p = 0.14 for right TLE, χ²; χ²(1) = 0.14, p = 0.91 for left TLE, and χ²; χ²(1) = 0.38, p = 0.54 for bilateral TLE].

3.2. Group comparisons (Tables 2 and 3)

Comparisons were made between right and left hippocampi and amygdalae values in patients groups and controls. In patients groups, values ipsilateral and contralateral to the focus were tested and the asymmetry indices (AI) were compared to control values.

In the control group, the only significant asymmetry was observed regarding the hippocampal volumes (right > left; p < 0.05). There were no gender differences in the hippocampal or amygdala volumes, and T2 and HCSI values. Also, there was no correlation between these values and age.

At a group level, these qMRI modalities presented some differences regarding the identification of amygdalo-hippocampal pathology, when compared with the control group.

In the groups with EEG focus lateralized either to the right or left TL, every qMRI analysis showed mean values that were significantly different to controls in the ipsilateral hippocampus and amygdala. HCVOL and AMYVOL did not have any significant changes (or trend) in the contralateral mean values relative to

### Table 2

Descriptive statistics for quantitative hippocampal MRI data (HCVOL, HCT2 and HCSI)

<table>
<thead>
<tr>
<th>Hippocampus</th>
<th>Controls (n = 34)</th>
<th>Right TLE (n = 45)</th>
<th>Left TLE (n = 39)</th>
<th>Non-lateralized (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>3604, 353 (1357–4518)</td>
<td>2640, 356 (1471–3901)</td>
<td>2640, 356 (1471–3901)</td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td>3463, 344 (2824–4310)</td>
<td>3387, 456 (2192–4180)</td>
<td>3387, 456 (2192–4180)</td>
<td></td>
</tr>
<tr>
<td>AI</td>
<td>4.3, 2.1 (0.8–7.5)</td>
<td>29.3, 16.3 (1.4–77.8)</td>
<td>35.9, 21.8 (0.2–78.7)</td>
<td>18.7, 15.8 (2.2–53.9)</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>83.2, 2.3 (79.0–88.4)</td>
<td>92.8, 5.6 (79.4–109)</td>
<td>84.8, 4.7 (72.6–100)</td>
<td>88.9, 6.7 (82.6–103.4)</td>
</tr>
<tr>
<td>Left</td>
<td>82.6, 2.5 (78.4–87.6)</td>
<td>85.6, 4.7 (76.4–108.6)</td>
<td>93, 7.4 (76.1–103.1)</td>
<td>91.2, 7.8 (78.5–101.5)</td>
</tr>
<tr>
<td>AI</td>
<td>1.8, 1.1 (0.2–3.3)</td>
<td>9.4, 5.1 (0.9–24.9)</td>
<td>9.5, 7.4 (0.3–27.7)</td>
<td>4.3, 5.4 (0.2–17.1)</td>
</tr>
<tr>
<td>NAA/(Cho + Cre)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>0.79, 0.06 (0.66–0.92)</td>
<td>0.56, 0.11 (0.33–0.79)</td>
<td>0.65, 0.13 (0.46–1.03)</td>
<td>0.57, 0.09 (0.48–0.73)</td>
</tr>
<tr>
<td>Left</td>
<td>0.78, 0.06 (0.64–0.88)</td>
<td>0.64, 0.09 (0.37–0.82)</td>
<td>0.54, 0.13 (0.34–0.80)</td>
<td>0.55, 0.13 (0.36–0.78)</td>
</tr>
<tr>
<td>AI</td>
<td>4.1, 3.5 (0.3–11.9)</td>
<td>16.2, 14.6 (1.5–69.6)</td>
<td>19.8, 11.5 (0.9–40.3)</td>
<td>10.9, 10.6 (2.1–29.3)</td>
</tr>
</tbody>
</table>

All values are expressed as mean; 1 standard deviation (range). AI, asymmetry indexes. Volume in mm³; T2 in ms; NAA/(Cho + Cre) in ppm.

1. Significantly different from the contralateral side at a level of 0.01.
2. Significantly different from the correspondent control side at a level of 0.01.
3. Significantly different from the contralateral side at a level of 0.05.
P.M. Gonçalves Pereira et al. / Epilepsy Research 69 (2006) 147–164

Table 3

Descriptive statistics for quantitative amygdala MRI data (AMYVOL and AMYT2)

<table>
<thead>
<tr>
<th>Amygdala</th>
<th>Controls (n = 34)</th>
<th>Right TLE (n = 45)</th>
<th>Left TLE (n = 39)</th>
<th>Non-lateralized (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>AI</strong></td>
<td>4.2, 2.6 (0.2–10.1)</td>
<td>11.4, 8.9 (0.5–34.1)**</td>
<td>11.9, 8.4 (0.3–35.7)**</td>
<td>12.2, 12.1 (0.4–34.1)†</td>
</tr>
<tr>
<td><strong>T2</strong></td>
<td>79.6, 2.9 (73.8–84.9)</td>
<td>86.8, 5.2 (76.1–98.1)**, ‡</td>
<td>80.5, 3.5 (73.5–87.8)</td>
<td>83.1, 6.4 (76.7–96.3)‡</td>
</tr>
<tr>
<td>Right amygdala</td>
<td>79.5, 2.4 (73.8–84.5)</td>
<td>82.1, 5.1 (72.1–93.9)</td>
<td>84.9, 4.9 (74.2–96.1)**, ‡</td>
<td>7.3, 6.6 (1.5–21.4)</td>
</tr>
<tr>
<td>Left amygdala</td>
<td>79.5, 2.4 (73.8–84.5)</td>
<td>82.1, 5.1 (72.1–93.9)</td>
<td>84.9, 4.9 (74.2–96.1)**, ‡</td>
<td>7.3, 6.6 (1.5–21.4)</td>
</tr>
<tr>
<td><strong>AI</strong></td>
<td>2.3, 1.5 (0.6–5.5)</td>
<td>6.7, 3.9 (0.3–15.8)**</td>
<td>6.1, 4.5 (0.2–17.1)†</td>
<td></td>
</tr>
</tbody>
</table>

All values are expressed as mean, 1 standard deviation (range). AI, asymmetry indexes. Volume in mm³; T2 in ms.

∗∗ Significantly different from the contralateral side at α level of 0.01.
‡ Significantly different from the correspondent control side at α level of 0.01.
† Significantly different from the correspondent control side at α level of 0.05.

controls. Moreover, HCVOL had the largest mean AI difference relative to controls (whose mean was 4.3), with values reaching a mean of 35.9.

The overall trend for HCT2, AMYT2 and HCSI showed changes in the structures that were contralateral to the focus, indicating that a certain number of patients with clear localized EEG focus and concomitant atrophy had widespread mesial abnormalities on both temporal lobes. HCT2 identified contralateral structural damage in the right TLE group, although there was a similar tendency for the left TLE group that was not significant (p = 0.06 for the HCT2 mean values of the right hippocampus). There was also an additional tendency in the right TLE group for large mean AMYT2 values in the left amygdala (p = 0.08). HCSI values from both hippocampi were significantly smaller than controls on both right and left TLE groups.

The non-lateralized patients showed significant mean bilateral differences compared with controls on every hippocampal and amygdala analysis, except for AMYT2. Furthermore, there were significant differences of the asymmetry indices of HCVOL (p < 0.05) suggesting that some of these patients would have pathology on a dominant side.

Since one main objective of this study was to determine the relative value of the qMRI methods to lateralize hippocampal and amygdala abnormalities, we present these data, for patients and controls, in two-dimensional plots (confidence ellipses) where the absolute values and the asymmetries can be put more clearly in evidence for the individual patients.

3.3. Confidence ellipses (Fig. 3 and Table 4)

The ellipses represent the 99% confidence interval for the control group. The specificity of the three qMRI methods was 100%, since no control subject had values outside the interval defined as normal.

The present data was analysed considering, first, whether the individual values were significantly asymmetric (which may indicate lateralization of pathology) and, second, identifying the patients with asymmetry indices in the same range as controls. The latter were either indistinguishable from controls or presented bilateral abnormalities (see the summary of data in Table 4).

3.3.1. Volumetry (Fig. 3A and B)

There was a clear tendency for significant asymmetries (or atrophies) in agreement with the EEG data, namely for HCVOL. Considering both TLE patients groups (n = 92), HCVOL (Fig. 3A) detected asymmetry in 77% (71/92) of the patients, 6% (5/92) had bilateral small volumes, while the remaining (17%, 16/92) had normal volumes and no asymmetries. AMYT2 (Fig. 3B) revealed that 38% (35/92) of the patients had an asymmetric volume, one patient had a bilateral larger AMYT2 and 59% (55/92) had normal volumes.

There were 39% (36/92) of the patients with concordant HCVOL and AMYT2 data [either pathological (n = 24) or normal (n = 12)], 43% (40/92) with an asymmetric HCVOL without AMYT2 changes,
156

P. M. Gonçalves Pereira et al. / Epilepsy Research 69 (2006) 147–164

and 5% (4/92) of the patients had isolated amygdala atrophies (there were 12 cases with discordant HCVOL–AMYVOL data, that is, either asymmetries on opposite sides or bilateral HCVOL atrophy with normal or asymmetric AMYVOL).

3.3.2. T2-relaxometry (Fig. 3C and D)

These cases indicate a general trend for T2-relaxometry to be concordant with the EEG data (and with volumetry), although it detects a larger number of cases with bilateral damage. Another interesting observation was that the number of cases with pathology detected by AMYT2 nearly reaches 50% more than the ones detected by AMYVOL, with less contralateral detections. The HCT2 showed that (Fig. 3C), 64% (59/92) of the patients had asymmetric T2 values, while 10% (9/92) had bilateral large T2 and the remaining 26% (24/92) were normal. AMYT2 data (Fig. 3D) showed that 45% (41/92) of the patients had an asymmetry index significantly larger than controls, 7% (6/92) had bilaterally large values and 49% (45/92) were normal.
### Table 4
Summary of data for quantitative hippocampal and amygdala MRI analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Group</th>
<th>Pathological AI (%) (ipsilateral–contralateral cases, %)</th>
<th>Normal AI (%) (normal–bilateral cases, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lateralized (n = 84)</td>
<td>82 (77–5)</td>
<td>18 (15–3)</td>
</tr>
<tr>
<td></td>
<td>Non-lateralized (n = 8)</td>
<td>38</td>
<td>62 (24–38)</td>
</tr>
<tr>
<td>HCVOL</td>
<td>Lateralized (n = 84)</td>
<td>69 (66–1)</td>
<td>31 (24–7)</td>
</tr>
<tr>
<td></td>
<td>Non-lateralized (n = 8)</td>
<td>12</td>
<td>88 (50–38)</td>
</tr>
<tr>
<td>HCT2</td>
<td>Lateralized (n = 84)</td>
<td>55 (53–2)</td>
<td>45 (27–18)</td>
</tr>
<tr>
<td></td>
<td>Non-lateralized (n = 8)</td>
<td>38</td>
<td>62 (24–38)</td>
</tr>
<tr>
<td>HCSI</td>
<td>Lateralized (n = 84)</td>
<td>37 (27–10)</td>
<td>61 (60–3)</td>
</tr>
<tr>
<td></td>
<td>Non-lateralized (n = 8)</td>
<td>50</td>
<td>50 (50–0)</td>
</tr>
<tr>
<td>AMYVOL</td>
<td>Lateralized (n = 84)</td>
<td>44 (43–1)</td>
<td>56 (49–7)</td>
</tr>
<tr>
<td></td>
<td>Non-lateralized (n = 8)</td>
<td>50</td>
<td>50 (50–0)</td>
</tr>
</tbody>
</table>

All values are expressed in percentage for the respective group. AI, asymmetry indices. Lateralized, patients with a right or left; EEG focus. Non-lateralized, patients without a definable side for the EEG focus.

* The ipsilateral–contralateral distinction does not apply to the non-lateralized patients.

* Two patients with a bilateral large AMYVOL.

There were 78% (72/92) patients with concomitant HCT2 and HCVOL data (either pathological or normal), 3% (3/92) patients had a bilateral large T2 value and unilateral hippocampal atrophy, and one patient had a bilateral large HCT2 with a normal hippocampal volume. In the amygdala, there were 48% (44/92) patients with concomitant AMYVOL data (either pathological or normal) and 4% (3/92) patients had a large T2 value and a normal amygdala volume.

Regarding the agreement between HCT2 and AMYVOL data, 47% (43/92) patients had concordant values (either pathological (n = 31) or normal (n = 12)) and 8% (7/92) patients had isolated large T2 values in the amygdala.

#### 3.3.3. Hippocampal spectroscopy (Fig. 3E)

HCSI showed a general trend towards low bilateral NAA/(Cho + Cre) ratios in all the TLE patients groups. In the EEG lateralized groups, nearly 50% of the patients had an AI in the same range as controls. Furthermore, 53% (49/92) of the patients had an asymmetric NAA/(Cho + Cre) ratio (ipsilateral HC > contralateral HC), 20% (18/92) had a bilaterally small ratio and 27% (25/92) were normal.

There were 53% (49/92) patients with concordant HCSI and HCVOL and 55% (51/92) patients with concomitant HCSI and HCT2 data (either pathological or normal). Other 5% (4/92) patients had isolated asymmetrical HCSI values.

#### 3.4. Relations between variables

Only values from the right and left TLE patients groups (n = 84) were included in this analysis. There were significant correlations among all ipsilateral MR variables, being the strongest association between HCVOL and HCT2 (r = -0.72, p < 0.01). The correlations between the contralateral values were non-significant, except for HCVOL versus HCT2 (r = -0.27, p < 0.01) and HCT2 versus HCSI (r = -0.25, p < 0.05).

The co-occurrence of amygdala and hippocampal damage was also assessed. There were significantly moderate positive correlations between hippocampal and amygdala ipsilateral volumes (r = 0.34, p < 0.01) and T2 values (r = 0.36, p < 0.01). As for the contralateral data, there was a weak positive association between the volumes (r = 0.26, p < 0.05) and a moderate correlation between the T2 values (r = 0.40, p < 0.05).

#### 3.5. Post-surgical outcome and qMRI

During the follow-up period, 21 (75%) patients were in class IA (seizure-free), and 7 (25%) in classes IB–III (3 patients in class IB, 3 in class II and 1 patient in class III). All 28 patients underwent surgery on the side of maximal EEG lateralization and had imaging features characteristic of hippocampal sclerosis on qualitative
Table 5  
Post-surgical outcome and relation with quantitative hippocampal and amygdala MRI analysis for the 28 patients operated

<table>
<thead>
<tr>
<th>Study</th>
<th>Lateralization</th>
<th>Favourable outcome (Engel I)</th>
<th>Unfavourable outcome (Engel II–III)</th>
<th>Positive predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCVOL</td>
<td>Concordant</td>
<td>20</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Discordant-lateralized</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>HCT2</td>
<td>Concordant</td>
<td>20</td>
<td>1</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>Discordant-lateralized</td>
<td>1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>HCSI</td>
<td>Concordant</td>
<td>14</td>
<td>2</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Discordant-lateralized</td>
<td>7</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>AMYVOL</td>
<td>Concordant</td>
<td>15</td>
<td>1</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>Discordant-lateralized</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>AMYT2</td>
<td>Concordant</td>
<td>16</td>
<td>1</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Discordant-lateralized</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Fisher exact test regarding a concordant lateralization to the side of major qMRI and EEG abnormalities and surgery.

\[ p < 0.05. \]

\[ * p < 0.01. \]

\[ ** p < 0.001. \]

MRI. No patient was operated based on isolated amygdala sclerosis imaging.

The association between the qMRI measures and a good surgical outcome was only demonstrated with respect to the volume and T2-relaxometry independently, and either in the hippocampus and amygdala (Table 5). Accordingly, significant negative \( r \) correlations were found between the volumes, and T2-relaxometry Asymmetry Indices (HCVOL: \( r_s = -0.73; \) HCT2: \( r_s = -0.71, \) both \( p < 0.0001; \) AMYVOL: \( r_s = -0.54, \) \( p < 0.01; \) AMYT2: \( r_s = -0.42, \) \( p < 0.05), \) with respect to the individual Engel class. Lateralized HCSI did not associate with outcome and Spearman's correlation was non-significant (\( r_s = -0.18, \) \( p = 0.36)).

Although the positive predictive value was rather similar among the volumes and T2-relaxometry of the hippocampus and amygdala (Table 5), there were major differences in the estimated odds ratio (OR). As both HCVOL and HCT2 provided identical results regarding lateralization and outcome, the OR was the same: 120 (95% CI 2.3–148.4). As for AMYVOL and AMYT2, the OR was 8 and 6 times smaller than that of the hippocampus, respectively (AMYVOL: OR = 15, 95% CI 3.2–33.4; AMYT2: OR = 19.2, 95% CI 2.9–36.9).

Binary logistic analyses were computed for the volumes and T2-relaxometry to verify whether successful surgical outcomes could be predicted accurately by qMRI covariates and whether stepwise regression models of the hippocampus and amygdala provided complementary or redundant information regarding patient counseling.

Models that used hippocampus-only covariates obtained the maximal accuracy concerning the correct classification of these patients. For the volume all the patients (100%) were exactly grouped (Nagelkerke \( R^2 = 0.99, \) Hosmer and Lemeshow test: \( p = 1), \) whenever the relation between the operated side (HCVOLPSI; units in mm\(^3\)), the non-operated side (HCVOLCONTRA; mm\(^3\)) and the volume asymmetry (HCVOLAI) holds:

\[
(0.01 \times \text{HCVOLPSI}) + (-0.08 \times \text{HCVOLCONTRA}) + (-4.53 \times \text{HCVOLAI}) + 333 < 0
\]

Interestingly, all the cases with an unfavourable outcome had a side-to-side difference (HCVOLCONTRA – HCVOLPSI) smaller than 900 mm\(^3\), a value that matches the cut-off limit found by Jack et al. (1992) on a larger study (50 patients; difference in volume <1000 mm\(^3\)).

As for HCT2, the model classified correctly 96.4% (27/28) of the patients (Nagelkerke \( R^2 = 0.83, \) Hosmer and Lemeshow test: \( p = 0.69)) whenever the T2 asym-
metry (HCT2AI) assumes the following assumption:

\((-0.91 \times \text{HCT2AI}) + 5.10 < 0\)

For every patient that was operated with an HCT2 value in the non-operated side larger than the HCT2 in the resected side (irrespective of the difference), the outcome was unfavourable.

The difference in accuracy between both models was effectively due to a single patient, who had a bilaterally larger HCT2 and clear-cut unilateral hippocampal volume atrophy, with “moderate HS” on histology and classified in class IA (at 24 months after surgery).

3.6. Post-surgical outcome and neuropathological data

Hippocampal sclerosis, as defined, was found in all surgical specimens.

Globally, 16 (57%) hippocampi abnormalities were classified as “severe HS”, 9 (32%) as “moderate HS” and 3 (11%) as end-folium sclerosis.

Of the 21 patients in class IA, 14 (66%) had a diagnosis of “severe HS”, 6 (29%) had “moderate HS” and 1 (5%) patient had end-folium sclerosis. Patients whose postoperative outcome was less favourable (classes IB–III, n = 7), had a neuropathological diagnosis of “severe HS” in two cases (29%), “moderate HS” in three cases (43%) and end-folium sclerosis in two cases (29%).

There was no significant association between the neuropathological data and surgical outcome, although a tendency could be depicted (Fisher’s exact test: \( p = 0.103 \); Spearman’s correlation: \( r_s = 0.38, p = 0.04 \)).

3.7. Neuropathological data and hippocampal qMRI

There were significant differences in the mean values of the asymmetry indices of HCVOL and HCT2, accordingly to the neuropathological classification. Patients with “severe HS” had larger AI than “moderate HS”; similarly, the “moderate HS” class had a larger AI than the end-folium class (HCVOLAI: \( p = 0.00001 \); HCT2AI: \( p = 0.04 \)). Furthermore, the mean value of the HCVOLPSI differed significantly across pathological classes, with smaller volumes in the “severe HS” class and larger volumes in the end-folium class (\( p = 0.02 \)).

Correlation analysis demonstrated a significant positive \( \rho \) between the HCVOLPSI (\( r_s = 0.62, p < 0.0001 \)), with respect to the individual pathological class, and negative \( \rho \) correlations between the volumes, and T2-relaxometry asymmetry indices (HCVOLAI: \( r_s = -0.76, p < 0.0001 \); HCT2AI: \( r_s = -0.48, p < 0.01 \)).

4. Discussion

Accurate lesion localization is vital in order to make anatomical inferences based on clinical and neurophysiological data and to support surgical decisions. In the present study, we used a multimodal set of quantitative MRI methods to assess the occurrence and severity of hippocampal and amygdala pathology in 92 TLE patients, 30% of which underwent amygdalohippocampectomy, and compare the lateralizing ability of each modality. We further aimed to propose a comprehensive qMRI protocol for the pre-surgical evaluation of refractory TLE patients, based on both the ability to detect mesio-temporal damage and time efficiency.

There were four main findings in this study.

First, regarding the hippocampus, volumetry and relaxometry detected the largest number of cases with unilateral damage that agreed with the EEG, with a strong correlation between them. Accordingly, in the 4 patients (5%) with contralateral volume loss, T2-relaxometry expressed either bilateral (3 patients) or contralateral (1 patient) pathologies. Still, T2-relaxometry identified less 13 patients (14%) with unilateral damage than volumetry. In the sub-group of patients that were operated, both HCVOL and HCT2 predicted outcome with 100% and 96.4% accuracy, respectively. Both measures were also associated with the severity of hippocampal sclerosis.

Second, hippocampal spectroscopy unveiled a large number of cases with bilateral abnormalities, although the majority was asymmetric, and yielded the largest number of cases with normal symmetric values when compared with the volume and T2 analyses. Additionally, HCSI was not a predictive variable with respect to the surgical results, neither correlated with the neuropathological results. These data suggest that HCSI is less precise in the identification of tissue damage and that it detects changes, other than structural, in the normal appearing hippocampus.
Third, in the amygdala, T2-relaxometry detected nearly 50% more pathological cases than volumetry and had less contralateral detections. Moreover, there was a complete concordance between cases with isolated amygdala damage: the four patients with amygdala atrophy were detected by T2-relaxometry, which, additionally, detected other three patients with pathology. Although significantly associated with the post-operative results, none of the amygdala measures contributed decisively to the predictive models that were generated regarding outcome.

Fourth, for diagnostic purposes the co-occurrence of amygdalo-hippocampal pathology was best assessed by the number of common cases identified by T2-relaxometry measures, which had also the highest level of correlation.

4.1. Lateralizing ability and technical considerations

Electroencephalography and qMRI data are independent sets of variables used to characterize different abnormalities from the area where seizures originate. Thus, concordance of the results provided by these analyses further validates the diagnostic conclusions derived from the individual studies. However, it must be realized that it is possible to encounter incongruent data between video-EEG and the newer imaging techniques and in different circumstances either one may be more indicative of the true epileptic focus.

Our first analysis revealed that the use of multiple qMRI modalities to study the hippocampus and the amygdala in patients evaluated for mesio-temporal surgeries offers advantages over individual modalities. The combined lateralizing ability of all five studies was 85% for the quantification of pathology in agreement with the EEG. However, complete concordance between all the hippocampal qMRI studies and the EEG was seen in 39% (36/92) and between both the amygdala qMRI studies and the EEG was verified in 15% (14/92). This indicates that some imaging sequences provide less than optimal sensitivity for MTS, either because they do not entirely associate with structural damage [e.g. hippocampal spectroscopy (Kuzniecky et al., 2001)] or due to its intrinsic variation and heterogeneity of measures despite using an optimal protocol [e.g. volumetry of the amygdala (Brierley et al., 2002)].

Our suggestion is that not all qMRI modalities are useful in the diagnosis of MTS and, thus, may increase the cost-effectiveness of the study.

Among all the qMRI modalities tested with this protocol, volume calculations using a manual segmentation method are the most time-consuming and are more prone to bias from the observer. In our hands, the total time for an amygdalo-hippocampal study with normalized volumes surpasses 1 h. In this respect, multi-voxel spectroscopy studies are less demanding. Nevertheless, it was the modality that detected less number of pathological cases and showed less lateralizing ability, and specifically not associated with the surgical results. Additionally, 2D spectroscopic analyses are restricted to the hippocampus, since amygdala analyses are difficult to implement.

Our findings that a large number of cases (47%) present a symmetric NAA/(Cho + Cre) ratio are not uncommon (Connelly et al., 1994). Thompson et al. (1998) found that only 42% of the TLE patients where effectively lateralized by the index of asymmetry of NAA/(Cho + Cre). Ende et al. (1997) described a surgical series of TLE patients in which 50%, although revealed decreased NAA and NAA/(Cho + Cre) levels in all ipsilateral temporal lobes had concomitant small metabolites values in the contralateral temporal lobe. Moreover, the patients who underwent surgery and had either a bilaterally small NAA value or normal NAA/(Cho + Cre) ratio (that is, an asymmetry index in range with control values) continued to have seizures. A similar trend has been observed by others (Duc et al., 1998; Kantarci et al., 2002), which emphasizes the value of MR spectroscopy in the prediction of surgical outcome. Our data indicates that spectroscopy cannot be used has a valid predictive measure for surgery success or has an adjuvant for localization. As such, further studies are needed to clarify the role of MR spectroscopy.

T2-relaxometry offers the advantage of studying both mesio-temporal structures with a single quantitative examination, which can be concluded with the present protocol in 25–30 min. The most relevant limitation of the T2-relaxometry analysis in our population was that it left undetected 14% of cases with an atrophic hippocampus and failed in the correct classification of one post-surgical case. However, a detailed analysis of these cases revealed that, although pathological, the degree of atrophy was smaller (range of AI: 17.9–38.1;
mean, 1 S.D. = 22.6, 9.6) when compared with the range of asymmetry for the overall patients (range up to 78.7; mean, 1 S.D. = 32.1, 19.3). Data from different laboratories (Van Paesschen et al., 1995; Kuzniecky et al., 1997) reported normal HCT2 maps in patients with what was considered a milder form of HS. These patients had a characteristic end-folium sclerosis, a similar trend with respect to our neuropathological data. Therefore, it is possible that HCT2 is less sensitive to milder forms of tissue damage than HCVOL. Moreover, mild forms of mesial temporal pathology may underlie surgically remediable epilepsy and not have any abnormality on MRI.

4.2. Relevance of amygdala studies

Intracerebral recordings from TLE patients (Kennedy and Hill, 1958; Wieser, 1991; Maldonado et al., 1998), usually implicate more than one mesio-temporal region on seizure onset, being the hippocampus and the amygdala commonly involved on both the onset and propagation of epileptiform activity. Amygdala sclerosis occurs mostly associated with HS, with up to 60% of the cases showing a histologically combined lesion (Cavanagh and Meyer, 1956; Bruton, 1988).

Our qMRI analysis demonstrated that T2-relaxometry performed better than volumetry in the identification of amygdala damage and showed higher level of association with the hippocampal lesion than volumetric studies. Although volumetric studies of the hippocampus bear a considerable bias among laboratories (Hasboun et al., 1996), volume analyses of the amygdala show highly significant variations (Brierley et al., 2002). Moreover, amygdala sclerosis occurs in a nuclei-specific form within the complex (rather than with a layer-specific distribution within the hippocampus), being the lateral nucleus most often involved than basal or granular nuclei (Pitkänen et al., 1998; Yilmazer-Hanke et al., 2000). Other studies also reported increased astrocytosis in the amygdala of epileptic patients (Hudson et al., 1993), which may

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**Fig. 4.** Proposed qMRI protocol for pre-surgical evaluation privileges T2-relaxometry as the single diagnostic method to study hippocampo-amygdala pathology, reserving hippocampal volumetry for a secondary analysis due to the excessive processing time. Whenever a surgical decision is reached, hippocampal volumetry can be performed since it guarantees a higher predictive value than T2-relaxometry. Hippocampal multivoxel spectroscopy and amygdala volumetry are excluded. HCT2, hippocampal T2-relaxometry; HCVOL, hippocampal volumetry; AMYT2, amygdala T2-relaxometry; PET, positron emission tomography.
underlie the prolonged T2 times observed by qMRI. A detailed examination of the amygdala sub-regional pathology reported recently by Yilmazer-Hanke et al. (2000) reveals that in the lateral nucleus, 45% of the specimens present severe pathology with neuronal loss and fibrillary astrogliosis (grade 4) while the remaining 55% show either moderate neuronal loss with mixed fibrillary and cellular astrogliosis (grade 3) or cellular astrogliosis without significant cell loss (grade 2). In the basal and granular nuclei, 23% showed a grade 4, 63% a grade 3–2 and 14% were normal specimens. Thus, there is a predominance of astrogliosis over neuronal cell loss, which may also explain why T2-relaxometry provides a larger detection of pathology than volumetry.

4.3. A comprehensive qMRI protocol for pre-surgical screening

Quantitative imaging has been recommended to be part of the whole pre-surgical evaluation of TLE patients (Sakamoto et al., 2001). One main objective of this study was to define a simple qMRI step-by-step protocol to be implemented on a diary routine. It must be noted that we are proposing it as a complementary tool to qualitative high-resolution MRI imaging, and performed on experienced centres.

This qMRI protocol (Fig. 4) was based on the necessity to study both the hippocampus and the amygdala on a time-efficient manner. As such, T2-relaxometry provided the adequate balance between scanning and processing times, on one hand, and the ability to uncover hippocampo-amygdala pathology, which agrees with the EEG and post-surgical data, on the other. However, hippocampal volumetry must be performed on selected patients to obtain maximal predictive accuracy with respect to surgical outcome.

Giving the number of patients that underwent surgery and the fact that some (n = 5) had a follow-up period of less than 2 years, the correlation between qMRI findings and outcome may need additional validation.

5. Conclusion

Previous work has indicated that approximately 20% of patients with TLE remain MRI negative after extensive qualitative imaging investigations. Such patients could benefit of additional quantitative MRI studies to study both the hippocampus and the amygdala.

For diagnostic purposes, of the three qMRI modalities tested, hippocampal T2-relaxometry provided an adequate balance between diagnosis accuracy and time-efficiency to lateralize a sclerotic lesion on the majority of the patients. Cases that are negative under this first analysis may benefit from further studies with hippocampal volumetry and amygdala T2s.

For pre-operative analysis and to maximally predict seizure freedom, hippocampal volumetry should be performed in all the patients.

Spectroscopy has limited utility to depict the structural abnormalities of the sclerotic hippocampus and in the pre-surgical evaluation of TLE patients.

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