Abnormalities of grey and white matter
$[^{11}C]$flumazenil binding in temporal lobe epilepsy
with normal MRI

A. Hammers,1,2,3 M. J. Koepp,1,2,3 R. Hurlemann,2 M. Thom,2 M. P. Richardson,1,2,3 D. J. Brooks1 and
J. S. Duncan1,2,3

1MRC Clinical Sciences Centre and Division of Neurosciences, Faculty of Medicine, Imperial College,
2Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London, London
and 3National Society for Epilepsy MRI Unit, Chalfont St Peter, UK

Correspondence to: Professor John S. Duncan, MA, DM, FRCP, National Society for Epilepsy and Institute of
Neurology, 33 Queen Square, London WC1N 3BG, UK
E-mail: j.duncan@ion.ucl.ac.uk

Summary
In 20% of potential surgical candidates with refractory epilepsy, current optimal MRI does not identify
the cause. GABA is the principal inhibitory neurotransmitter in the brain, and GABAA receptors are
expressed by most neurons. $[^{11}C]$Flumazenil (FMZ) PET images the majority of GABAA receptor sub-
types. We investigated abnormalities of FMZ binding in grey and white matter in 18 patients with refrac-
tory temporal lobe epilepsy (TLE) and normal quantitative MRI. Parametric images of FMZ volume of
distribution (FMZ-Vd) were calculated. Twenty-one healthy controls were scanned for comparison.
Statistical parametric mapping (SPM99) was used to localize significant changes in FMZ-Vd in individual
patients and between groups, specifically including the entire white matter in all subjects through expli-
cit masking. Sixteen of 18 patients showed single or multiple abnormalities of FMZ-Vd. Six had hippo-
campal decreases of FMZ-Vd. Eleven patients showed increased FMZ-Vd in the temporal lobe white matter
(TLWM). Outside the mesial temporal structures, seven showed multiple areas of increase or decrease
and only one a single area of decrease. In seven of the 16 patients with abnormalities, findings were con-
cordant with EEG and clinical data, enabling further presurgical evaluation. Group findings were: (i)
decreased FMZ-Vd in the ipsilateral ($Z = 3.01$) and contralateral ($Z = 2.56$) hippocampus; (ii) increased
FMZ-Vd in the ipsilateral ($Z = 3.71$) and contralateral TLWM (two clusters, $Z = 3.11$ and 2.79); and
(iii) increased FMZ-Vd in the ipsilateral frontal lobe white matter between the superior and medial frontal
gyrus ($Z = 3.80$) with similar changes contralaterally ($Z = 4.87$). No changes were found in the thalamus
and basal ganglia. Region-of-interest analyses indicated an average increase in FMZ binding of 16%
in the TLWM ipsilateral to the epileptic focus. PET findings were corroborated by invasive EEG or pathol-
yogy in five cases. FMZ–PET, analysed by SPM with explicit masking, was sensitive in patients with nor-
mal MRI, and hippocampal abnormalities were detected in a third of these patients. Furthermore,
increases in FMZ binding in TLWM, indicating microdysgenesis, were detected in the majority of
these patients and may represent the structural basis of their epilepsy.

Keywords: PET; SPM99; temporal lobe epilepsy; flumazenil binding

Abbreviations: FLWM = frontal lobe white matter; FMZ = flumazenil; FMZ-Vd = flumazenil volume of distribution;
FWHM = full width half maximum; SPM = statistical parametric mapping; TLE = temporal lobe epilepsy; TLWM =
temporal lobe white matter

Introduction
In patients with medically refractory partial seizures, surgery offers the possibility of lasting suppression of
seizures. The majority of patients referred for epilepsy surgery have temporal lobe epilepsy (TLE), and 60% of
these have hippocampal sclerosis (Babb et al., 1984). MRI has been reported to be normal in 15–30% of
patients with TLE, even when histopathological examination of resected specimens detects hippocampal sclerosis, focal cortical dysplasia or other pathologies (Chugani et al., 1990; Kuzniecky et al., 1991; Desbiens et al., 1993; Van Paesschen et al., 1997). In the absence of identifiable pathology on imaging, surgery in TLE patients has a less favourable outcome (Berkovic et al., 1995). Thus, while patients with TLE and normal MRI represent a relatively large and important group in epilepsy centres, they are less likely to undergo surgery.

γ-Aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain, acting at the GABAA receptor complex. Flumazenil (FMZ) is a specific, reversibly bound high-affinity neutral antagonist at the benzodiazepine site of the GABAA receptor (Olsen et al., 1990), expressed by most neurones. [11C]FMZ provides a useful in vivo marker of GABAA receptor binding (Maziere et al., 1984).

In TLE, we have shown, using partial volume effect-corrected FMZ-PET, that FMZ binding is reduced over and above hippocampal volume loss in hippocampal sclerosis (Koepp et al., 1997b). Correlation analysis of autoradiography and quantitative neuropathology in resected hippocampi also revealed a greater reduction in FMZ binding than in neuronal cell density (Hand et al., 1997) and good correlation between in vivo [11C]FMZ-PET and [3H]FMZ autoradiography (Koepp et al., 1998). These results suggest that [11C]FMZ-PET may be more sensitive than MRI in the identification of subtle hippocampal abnormalities, and therefore clinically useful when MRI is unremarkable.

Malformations of cortical development are increasingly recognized as underlying medically intractable epilepsy (Kuzniecky and Jackson, 1998). Microdysgenesis, a minimal form of malformation of cortical development, is not detectable on conventional MRI and a common form is an increased density of heterotopic neurones in the white matter (Raymond et al., 1995). We have recently demonstrated a strong and highly significant correlation between in vivo temporal lobe white matter (TLWM) FMZ binding and white matter neurone number in patients with hippocampal sclerosis who underwent anterior temporal lobe resection (Hammers et al., 2001a). FMZ-PET is uniquely suited to the study of microdysgenesis in white matter due to its highly specific binding to neurones and the resulting high contrast-to-noise ratio. Previous PET studies of patients with TLE and normal MRI have not considered FMZ binding to GABAA receptors in the white matter.

The aims of the present study were to: (i) determine the ability of FMZ-PET to localize abnormalities in TLE patients with normal, high-resolution, quantitative MRI, using a more advanced version of statistical parametric mapping (SPM99) than previously (Koepp et al., 2000) in a different and larger series of patients; (ii) investigate with SPM99 whether TLWM FMZ binding in these patients is increased, using explicit masking; and (iii) assess common abnormalities using group comparisons.

### Material and methods

#### Patients and controls

We studied 18 patients (11 women) with medically refractory TLE. The diagnosis and focus lateralization was based on a comprehensive assessment. This included seizure semiology obtained from eye witnesses or video recordings, interictal EEG abnormalities in all, ictal video-EEG findings in 10 patients (Table 1) and detailed neuropsychological assessment. The patients were recruited from the epilepsy clinics of the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK, and the National Society for Epilepsy, Chalfont St Peter, UK. The median age at onset of habitual seizures was 19 years (range 7–54 years), the median duration of epilepsy before the PET examination was 13 years (range 1–34 years) and the median age at PET examination was 37 years (range 17–64 years). No patient had a history of prolonged febrile convulsions. Patients had a median of 62 complex partial seizures per year (range 4–1270). The antiepileptic medications were lamotrigine (11 patients), phenytoin (7), carbamazepine (5), topiramate (3), vigabatrin (2), sodium valproate (2) and gabapentin (1). Most patients received a combination of two antiepileptic drugs; one patient was not taking any medication, two were on lamotrigine monotherapy and one was on carbamazepine monotherapy. Patients who were treated with benzodiazepines or barbiturates within 2 months of the PET examination were not included in the study as these drugs could possibly interfere with [11C]FMZ binding. Vigabatrin has been shown not to influence benzodiazepine binding (Verhoeff et al., 1999; Hammers et al., 2001b).

Twenty-one healthy volunteers (three women) were studied for comparison. The median age at examination was 31 years (range 20–71 years). They had no history of neurological or psychiatric disorder, were on no medication and had normal MRI studies.

Individuals did not consume alcohol within the 48 h preceding PET.

Written informed consent was obtained in all cases according to the Declaration of Helsinki. Approval was obtained from the Joint Ethics Committee of The Institute of Neurology and The National Hospital for Neurology and Neurosurgery, the Ethics Committee, Imperial College, Hammersmith Hospital and the UK Administration of Radiation Substances Advisory Committee (ARSAC).

Clinical data for all 18 patients are shown in Table 1. At the time of writing, four patients have undergone epilepsy surgery (Patients 8, 15, 17 and 18) and one (Patient 7) has been evaluated with depth electrodes.

#### PET

We used the same acquisition technique as described previously (Hammers et al., 2002). PET scans were performed in 3D mode with the septa retracted, using a 953B Siemens/CTI (Knoxville, TN, USA) PET camera with a
PET image analysis

$[11\text{C}]$FMZ-V$_d$ images were analysed using SPM99 (Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab (Mathworks, Sherborn, MA, USA). The images were volumetrically normalized to a symmetrical reference FMZ-V$_d$ template, created in our unit, that occupies the same space as the SPM99 MRI templates. The normalization procedure involves a linear 3D transformation and uses a set of smooth basis functions that allow for normalization at a finer anatomical scale (Ashburner and Friston, 1999). Images were smoothed using a $12 \times 12 \times 12$ mm FWHM isotropic Gaussian kernel as a final preprocessing step. This spatial filter accommodates inter-individual anatomical variability and improves the sensitivity of the statistical analysis (Friston et al., 1991). Each subject’s MRI scan was co-registered with his or her FMZ-V$_d$ image (Woods et al., 1993; Ashburner and Friston, 1997) and then transformed into standard space using the transformation matrix derived from the spatial normalization of that individual’s FMZ-V$_d$ image.

Statistical analysis

Significant differences between patients and control subjects were estimated according to the general linear model at each and every voxel of the normalized and smoothed images (Friston et al., 1995b). Parametric images of FMZ-V$_d$ were interrogated with SPM99 implemented in Matlab5 (Mathworks). Statistical parametric maps are 3D projections of statistical functions that are used to characterize significant regional differences in imaging data. We have described the use of SPM in $[11\text{C}]$FMZ PET studies of patients with unilateral hippocampal sclerosis (Koepp et al., 1996), in patients with malformations of cortical development (Richardson et al., 1996) and in patients with TLE and normal MRI (Koepp et al., 2000). SPM99 combines the general linear model to create the statistical map and random field theory to make statistical inferences about regional effects (Friston et al., 1995b; Worsley et al., 1996).

Individual patients were compared with the 21 normal control subjects using a design matrix designating global cerebral FMZ-V$_d$ differences as a confounding covariate (Friston et al., 1990). For the purposes of between-group statistical analysis, the $[11\text{C}]$FMZ-V$_d$ images of the six patients with right-sided TLE were reversed before spatial manipulation so that the focus was on the same (left) side in all patients. The three patients with bilateral foci (Table 1) were not included in the group analyses. Linear contrasts were used to test the hypotheses for specific focal effects. The resulting set of voxel values for each contrast constituted a statistical parametric map of the $t$ statistic ($\text{SPM}(t)$). The $\text{SPM}(t)$ were thresholded at $P = 0.001$ uncorrected. The significance of foci of relative FMZ-V$_d$ changes is estimated using random field theory, correcting for multiple comparisons using the number of resolution elements (resels) in the
<table>
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<tr>
<th>Patient</th>
<th>Age (years)/sex</th>
<th>Handedness</th>
<th>Onset of epilepsy (years)</th>
<th>Duration (years)</th>
<th>CPS/yr</th>
<th>Interval last CPS to PET (days)</th>
<th>Medication</th>
<th>EEG (interictal)</th>
<th>EEG (ictal)</th>
<th>Focus*</th>
<th>FMZ–PET: temporal increases</th>
<th>FMZ–PET: temporal decreases</th>
<th>FMZ–PET: extratemporal abnormalities (T/H)</th>
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<table>
<thead>
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<th>Patient</th>
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F = female, M = male, CPS = complex partial seizures, EEG = electroencephalography, N/A = not available, HC = hippocampus, LTG = lamotrigine, PHF = phenytoin, CR2 = carbamazepine, TPM = topiramate, GVG = vigabatrin, VPA = valproic acid, GBP = gabapentin, Bil = bilateral, L = left, R = right, contralat = contralateral, ipsilat = ipsilateral, temp = temporal, amyg = amygdala, GM = grey matter, WM = white matter, TLW = temporal lobe white matter, G = gyrus temporalis superior, GTM = gyrus temporalis medius, FL = frontal lobe, Z = Z-score compared with 21 controls, k = number of voxels in cluster. Coordinates in brackets are MNI coordinates, referring to our symmetrical FMZ template which is in register with the MNI/ICBM152 T1 template as supplied with SPM99. *Focus determination was based on comprehensive assessment including seizure semiology, interictal and ictal Video-EEG findings where available as well as detailed neuropsychological assessment. **Patient underwent operation; histopathology available (see text).
statistical image (Worsley et al., 1992, 1996). This looks at the probability that the observed cluster of voxels could have occurred by chance, given its extent and peak height. The threshold chosen for the corrected $P$ values of the extramesial clusters was $P < 0.05$. As the hippocampus, amygdala and temporal lobe white matter are small structures, below twice the FWHM of our scanner resolution, FMZ-$V_d$ differences in these regions of special a priori interest were regarded as significant if the value of the $t$ statistic, transformed to the unit normal distribution, exceeded a $Z$-score of $>2.5$, as in our previous studies (Koepp et al., 1997a, b, 2000; Hammers et al., 2001a). Age, gender, age at onset of epilepsy, duration of epilepsy, frequency of seizures, time between scan and last seizure and hippocampal volume were defined as covariates of interest and tested separately for their effect on FMZ-$V_d$.

The aim was to localize abnormalities of FMZ-$V_d$ in individual patients compared with the control group and in the control group compared with the patient groups, in both grey and white matter. In SPM analyses the white matter, as a region of relatively low signal, is usually excluded by thresholding. This is done to include all areas of high signal while restricting the number of multiple comparisons by exclusion of areas that are not of interest in blood flow studies. To specifically include the entire white matter in the statistical analysis of all subjects, we therefore created an anatomical mask in template space, encompassing the grey matter in the cortex, the basal ganglia and the white matter.

In order to demonstrate the effect size, quantitative region-of-interest analyses were performed on non-normalized parametric images in the ipsilateral TLWM. An average of 2235 mm$^3$ was sampled with circular regions of interest, using Analyze AVW software (Robb and Hanson, 1991) in all 15 patients with a unilateral focus, and in corresponding areas in all 21 controls.

**Results**

**Controls**

**Hippocampus, amygdala and TLWM**

When individual control subjects were compared against the remaining 20 controls, none had FMZ-$V_d$ abnormalities in the white matter, hippocampus or amygdala.

**Extramesial structures**

One individual had an increase in the anterior pallidum ($Z = 4.67$, $k$ (number of voxels in cluster) = 390, $P = 0.003$) and another had increases in FMZ-$V_d$, localized to the left middle frontal gyrus ($Z = 4.30$, $k = 288$, $P = 0.011$) and the right orbitofrontal gyri ($Z = 4.25$, $k = 278$, $P = 0.013$).

Given the chosen thresholds and the number of comparisons resulting from 21 controls and two contrasts, two positive results would be predicted by chance.

**Individual patients (Table 1)**

**Hippocampus and amygdala**

Six patients had abnormally low FMZ-$V_d$ in the hippocampus. In three of them (Patients 8, 10 and 11) the decrease was ipsilateral to the EEG focus; one of these patients (Patient 10) had additional extramesial increases and one (Patient 11) had an additional TLWM increase (see below). Two others (Patients 7 and 17) had bilateral decreases and the sixth (Patient 6) had a unilateral decrease but bilateral EEG abnormalities. The decreases extended into the amygdala in Patients 8 (Fig. 1), 7 and 17. No increases in hippocampal or amygdala FMZ-$V_d$ were observed.

**Temporal lobe white matter**

Eleven of the 18 patients had FMZ-$V_d$ increases in the TLWM. In four patients they were ipsilateral, in two contralateral, in four bilateral and in one unilateral with bilateral EEG changes. Of the four patients with ipsilateral TLWM increases (Patients 1, 11, 12 and 16), three had additional abnormalities, an ipsilateral hippocampal decrease in one (Patient 11), multiple neocortical decreases in one (Patient 1) and an ipsilateral fronto white matter increase in another (Patient 16). Of the two patients with contralateral TLWM increases (Patients 4 and 18), one (Patient 4) also had a contralateral temporal neocortical increase. Of the four patients with bilateral TLWM increases (Patients 3, 5, 10 and 15) (Fig. 2), three had additional abnormalities: a contralateral frontal increase in one (Patient 3), an ipsilateral temporal neocortical decrease in another (Patient 15) (Fig. 3) and multiple white matter increases in one (Patient 10; see below).

**Extramesial structures**

Seven patients showed abnormalities outside the mesial temporal structures. Only one patient (Patient 9) had extramesial abnormalities only (increases in the orbitofrontal white and grey matter bilaterally); the other six had extramesial abnormalities in addition to their temporal lobe changes described above. Extramesial decreases were found in two patients: Patient 15 (Fig. 3) had a single temporal neocortical decrease and bilateral TLWM increases; Patient 1 had decreases in the ipsilateral temporal neocortex and ipsilateral frontal lobe (FL) in addition to ipsilateral TLWM increases. Extramesial increases were found in five patients. Three had one extramesial increase only: Patient 3 in the ipsilateral FL (in addition to bilateral TLWM increases), Patient 4 in the contralateral posterior TL (in addition to a contralateral TLWM increase) and Patient 16 in the ipsilateral FL white matter (in addition to the ipsilateral TLWM increase). Two had multiple extramesial increases. Patient 9 had bifrontal increases. Patient 10 had multiple white matter increases inferolaterally of both posterior temporal horns and
in both FLs, in addition to bilateral TLWM increases and an ipsilateral hippocampal decrease.

Between-group analyses
Hippocampus/amygdala
There was a decrease in FMZ-V_d in the ipsilateral hippocampus in the patient group, extending into the amygdala ($Z = 3.01$), as well as in the contralateral hippocampus ($Z = 2.56$) (Fig. 4).

Temporal lobe white matter
The patient group showed ipsilateral ($Z = 3.71$) and contralateral (two clusters, $Z = 3.11$ and $Z = 2.79$) increases in FMZ-V_d (Fig. 4).

In order to demonstrate the magnitude of the effect, quantitative region-of-interest analyses were performed on non-normalized parametric images. The ipsilateral TLWM was sampled in all 15 patients with a unilateral focus and corresponding areas were sampled in all 21 controls. These showed an average increase in FMZ-V_d of 16% in ipsilateral TLWM in the patients, which was highly significant (two-tailed $t$ test, $P < 0.007$).

Extramesial structures
We found increased FMZ-V_d in the ipsilateral frontal lobe white matter (FLWM) between the superior and medial frontal gyrus ($Z = 3.80$, $k = 293$, $P < 0.025$), with similar changes contralaterally ($Z = 4.87$, $k = 510$, $P < 0.0004$). There were no decreases outside the mesial temporal lobe.

Correlations with clinical data
There were no correlations anywhere in the brain between FMZ-V_d and age, gender, age at onset of epilepsy, duration of epilepsy, frequency of seizures, time between scan and last seizure, or hippocampal volume.

Surgery and histological results
So far, four patients have had surgery. One patient (Patient 15), with a marked unilateral temporal neocortical decrease in FMZ-V_d in addition to bilateral TLWM increases, has had a focal cortical resection. Histology showed a small malformative lesion with some features similar to Taylor-type focal cortical dysplasia (Taylor et al., 1971). There were dysplastic neurones in the cortex and gliosis. The demarcation between the grey and white matter was poorly defined. There were abnormal, large neurones present in the white matter, but only a small amount of white matter was resected and neuronal density could not be quantified. The patient remains seizure-free 25 months after the operation.

Three patients (Patients 8, 17 and 18) underwent modified anterior temporal lobe resections. One patient (Patient 8), with a left hippocampal decrease in FMZ-V_d extending into the amygdala, had a left anterior temporal lobe resection including the amygdala and hippocampus at another centre. Unfortunately, tissue was not preserved for detailed histological analysis and the hippocampal formation could not be identified in the resection specimen. The resected cortex showed moderate subpial and white matter gliosis. The patient remains seizure-free 26 months after the operation but has suffered a significant decline in verbal memory. Patient 17 had an ipsilateral (right) decrease in FMZ binding in the hippocampus and amygdala. A decrease in contralateral hippocampal FMZ binding was less marked in terms of both $Z$ score and spatial extent. Pathology showed amygdala gliosis (Fig. 5C and D) and mild end folium sclerosis (Fig. 5E). Immunohistochemistry with neuronal nuclear antigen (NeuN) and quantification showed low neuronal density in the TLWM (Fig. 5B) ($1010/\text{mm}^3$; control mean $1660 \pm 772 \text{ mm}^3$ (Thom et al., 2001)). The patient has been seizure-free for 4 months after the operation but recently had a single seizure within two weeks of starting fluoxetine. Patient 18 had no ipsilateral (right) abnormalities on FMZ PET but contralateral increases in FMZ binding in TLWM. On pathology, the right hippocampus was not fully represented, but parts of CA1, CA4 and the subiculum were available. There was mild gliosis but no evidence to support classical hippocampal sclerosis. TLWM neuronal density on quantitative immunohistochemistry with NeuN was moderately high (Fig. 5A) at $2368/\text{mm}^3$. The patient currently remains seizure-free 4 months after the operation.

One patient (Patient 7) with bilateral decreases in hippocampal FMZ-V_d, more marked on the left, has been evaluated with bilateral hippocampal depth electrodes. Nine
seizures were recorded; five started within the right and four within the left hippocampus.

Discussion
Patients with TLE and normal high-resolution MRI represent a challenge in epilepsy surgery referral centres. This is the largest study of such a group so far and the first to examine white matter changes in FMZ-Vd explicitly in these patients. The main novel finding is the high proportion (61%) of patients with increased FMZ binding in the white matter. In total, we found abnormalities in 16 out of 18 patients with TLE and normal high-resolution MRI, which included volumetric hippocampal studies and hippocampal T2 quantitation, confirming that [11C]FMZ PET detects functional abnormalities over and above the structural abnormalities revealed by optimal MRI (Koepp et al., 1996, 2000; Hammers et al., 2001a) analyses. In this study, we only included patients whose MRI was normal on inspection and in whom quantitative analysis of the hippocampi, including volumetry and T2 mapping, was entirely normal. In this situation, SPM is superior to region-based analyses as the entire brain volume can be studied and no assumptions about the number, localization and extent of abnormalities need to be made. Interpolation and smoothing necessarily decrease the spatial resolution of the resulting statistical map to ~13 mm FWHM. In contrast, the final spatial resolution of region-based analyses depends on the number and size of regions chosen (typically 10–50 for a given brain imaging data set). SPM examines the data at our level of smoothing with ~500 independent comparisons and without a priori hypotheses about the exact localization of neocortical abnormalities.

SPM does, however, need rigorous correction for the multiple comparisons made in order to prevent spurious false positives. If such a correction is applied to the entire volume, mesial temporal abnormalities, which are small in extent, may remain undetected. For mesial temporal structures (hippocampus and amygdala) and temporal white matter, we had a priori hypotheses. Within this defined small search volume (approximately four resolution elements), we did not correct for the spatial extent of the clusters of abnormal FMZ binding, but only for height, as in our previous studies (Koepp et al., 1997a, b, 2000; Hammers et al., 2001a). The validity of this approach was tested by investigating each control against the remaining 20 controls, and no mesial temporal false positives were detected. This rigorous exclusion of detection of artefactual increases or decreases amounts to a random effect analysis on our normal material.
In a previous study, we compared 10 different patients with normal quantitative MRI whose scans were acquired in temporal lobe orientation with 13 different controls with MRI acquired in temporal lobe orientation, using both an earlier version of SPM (SPM96) and partial volume effect correction (Koepp et al., 2000). Although FMZ-V$_d$ abnormalities were shown in eight of 10 patients, unilateral decreases were concordant with EEG data in only two, and most TLWM was excluded from the analysis by the use of standard thresholding methods.

The larger control group in this study makes the analysis more sensitive. Furthermore, the process of spatial normalization has been much improved in SPM99 (Ashburner and Friston, 1997, 1999). Better registration, particularly of the small mesial temporal structures, contributes to increased sensitivity and specificity.

We previously found a strong correlation ($r > 0.8$, $P < 0.001$) between preoperative white matter FMZ-V$_d$ in both temporal lobes and the white matter neurone number determined in the resected ipsilateral specimen of patients with hippocampal sclerosis (Hammers et al., 2001a). Increased TLWM neurone number is a hallmark of microdysgenesis. In this study, we investigated the hypothesis that microdysgenesis, manifesting itself as increased TLWM FMZ-V$_d$, could underlie some of the cases of TLE and normal MRI. SPM was originally devised for the study of grey matter, and thresholding normally excludes white matter from the analysis. Lowering this blanket threshold to include white matter signal gives inconsistent results due to the interindividual variability in global binding and increases the number of voxels included in the analysis, thereby accentuating the multiple comparisons problem. We therefore created an anatomical mask in template space, encompassing the grey matter in the cortex and basal ganglia as well as the central white matter. This enabled us to study explicitly, for the first time, white matter changes while restricting the number of voxels inspected. There is a theoretical concern that the error distribution could become positively skewed in areas of low binding due to the non-negativity constraint. The actual values derived from region-of-interest studies, however, indicate that mean values were more than 10 standard deviations away from zero in the TLWM, and the error distribution can therefore be approximated by parametric measures of variability.

**Neurobiological considerations**

There is now extensive evidence that decreased FMZ binding can localize the epileptogenic focus both in mesial TLE and in neocortical epilepsy (Savic et al., 1988, 1993, 1995; Henry et al., 1993; Koepp et al., 1996, 1997b; Szélies et al., 1996; Ryvlin et al., 1998; Muzik et al., 2000).

In earlier studies that included both patients with hippocampal sclerosis and patients with normal MRI, there was a clear negative correlation between hippocampal volume and FMZ binding (Henry et al., 1993; Lamusuo et al., 2000). Hippocampal FMZ binding is, however, reduced over and above volume loss in hippocampal sclerosis (Henry et al., 1993; Koepp et al., 1997a, b; Hammers et al., 2001a), indicating functional abnormalities over and above evident structural abnormalities. Our results suggest that the same is true for TLE with normal quantitative MRI: both the group of patients and six out of 18 individual patients showed a significant decrease in hippocampal FMZ-V$_d$ despite normal quantitative MRI. Moreover, there was no correlation between hippocampal volume and hippocampal FMZ-V$_d$, further indicating that our results were not due to atrophy or partial volume effect.

FMZ binds with high affinity to the benzodiazepine binding site of GABA$_A$ receptors containing $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits, and therefore our study can only look at the sum of effects of changes in these subunits. TLE is associated not only with changes in GABA$_A$ receptor number but also with differential changes in subunit composition (Brooks-Kayal et al., 1998; Sperk et al., 1997; Loup et al., 2000) that may precede the development of epilepsy in rats (Brooks-Kayal et al., 1998). With the development of subunit-specific tracers, it may be possible in the future to study epileptogenesis in vivo.

We found increases in FMZ-V$_d$ in the TLWM in 11 out of 18 patients with TLE and normal MRI. We suggest that these are due to microdysgenesis, i.e. an increased number of neurones in the white matter. There is a large body of direct and indirect evidence to support this claim. (i) We have previously shown a strong correlation between TLWM FMZ-V$_d$ and histologically determined neurone number in the white matter in patients with hippocampal sclerosis (Hammers et al., 2001a). (ii) In the three patients (Patients 15, 17 and 18) in the present series for whom TLWM was available for pathology, the prediction about the presence or absence of heterotopic neurones based on FMZ PET was confirmed and was quantified immunohistochemically with...
NeuN in two cases (Patients 17 and 18), directly validating our results. (iii) Increases in FMZ-Vd were frequently seen in malformations of cortical development (Richardson et al., 1996, 1997) but not in acquired lesions (Richardson et al., 1998). (iv) Histopathological studies of malformations of cortical development in humans have frequently shown increased white matter neurone numbers (Burdette et al., 1995; Battaglia et al., 1996; Spreafico et al., 1998; Hannan et al., 1999).

Theoretically, an increase in TLWM FMZ binding could be due to an apparent, relative rather than absolute increase in heterotopic neuronal density due to atrophy. We do not believe this can explain our results for the following reasons. (i) After the electroclinical diagnosis of TLE had been made, all MRIs were reviewed carefully with particular attention to the temporal lobes. No abnormality was found on inspection by experienced neuroradiologists or in either the quantitative volumetric studies or the T2 quantification studies of the hippocampi. The MRIs can therefore be regarded as normal by best current standards (Commission on Neuroimaging of the International League Against Epilepsy, 1997). (ii) Even minimal temporal lobe atrophy will be detected by SPM as a widespread decrease in FMZ binding along the border of the temporal neocortex (Hammers et al., 2001a), and this was not seen in this study. (iii) Neuronal density in the white matter does not correlate with the 3D volume of the white matter (Bothwell et al., 2001) or white matter gliosis (Kasper et al., 1999; Thom et al., 2001).

FMZ–PET is well suited to reflect abnormal neuronal density in white matter in vivo. Most neurones express GABA_A receptors and FMZ can, therefore, be regarded to a certain extent as a neuronal marker. Whereas clusters of heterotopic neurones need to be at least 1 mm^3 in size to be evident on MRI, the signal-to-noise ratio for heterotopic neurones in the white matter is very high for PET. The highly specific nature of FMZ binding makes it very improbable that

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**Fig. 5** (A and B) White matter neurones labelled with neuronal marker NeuN in Patient 18 (A) and Patient 17 (B), which demonstrated a lower number of both large and small neurones in a similar size field in B than A. (C) Luxol fast blue-stained section of the amygdala resection from Patient 17 shows some preservation of neurones, but marked diffuse gliosis is seen in the adjacent section immunostained for GFAP (glial fibrillary acidic protein) (D). (E) GFAP immunostaining in Patient 17 also demonstrated marked gliosis in the end folium (lower half of the field) extending into the granule cell layer and compatible with the diagnosis of end folium sclerosis. Bars = 50 μm.
these findings are functional in nature, in contrast to some abnormalities in grey matter FMZ binding (Ryvlin et al., 1999) or metabolic ([18F]fluorodeoxyglucose–PET) imaging.

Heterotopic white matter neurones can contribute to epileptogenesis by providing additional or abnormal circuitry. This has been shown experimentally for heterotopic CA1 neurones in the rat model of methylazoxymethanol-induced cortical malformations (Chevassus-au-Louis et al., 1998). The finding of heterotopic white matter neurones in TLE with normal MRI can, therefore, be a reasonable explanation for the pathogenesis of epileptic circuits in the face of normal structural imaging studies. The relevance of our findings for TLE is further underlined by the fact that 11 out of 18 patients but 0 out of 21 controls showed TLWM increases (χ² test, P < 0.0001).

Taken as a group, the patients showed significant increases in FMZ-Vd in the FLWM bilaterally. Widespread disturbances of cerebral structure have been demonstrated in malformations of cortical development (e.g. Richardson et al., 1997). The most tenable explanation for these increases in FMZ-Vd in the white matter is again the presence of heterotopic white matter neurones. We do not, however, have histological material to prove this. Moreover, in the individual analysis, FLWM increases in FMZ-Vd at a location closely related to that of the group finding were found in only one patient (Patient 10), who had very widespread white matter increases. It would be reasonable to assume that a diffuse disturbance of migration can manifest itself principally in the TLWM but have some smaller effect elsewhere. Frontal lobe white matter contains approximately six times fewer neurones in controls than TLWM (Rojiani et al., 1996), and our finding is compatible with a small effect size which reaches significance in SPM due to its large spatial extent and the large number of subjects studied.

Clinical considerations
Patients with normal or non-diagnostic MRI and medically refractory TLE are an important and difficult subgroup of patients who undergo investigations for epilepsy surgery. Accordingly, there have been various studies using FMZ–PET centred on or including ‘MRI-negative’ TLE patients (Henry et al., 1993; Savic et al., 1993; Szélies et al., 1996; Debets et al., 1997; Ryvlin et al., 1998; Koepp et al., 2000; Lamusuo et al., 2000). In most earlier studies, however, MRI was only assessed qualitatively for signs of hippocampal sclerosis, and quantitative hippocampal MRI data were only available in three studies (Ryvlin et al., 1998; Koepp et al., 2000; Lamusuo et al., 2000). Also, most of the earlier studies relied on measures of asymmetry for both hippocampal volumes and FMZ binding and could not therefore detect bilateral abnormalities.

In our series, amongst other findings, there were patients with hippocampal decreases in FMZ binding, most likely representing hippocampal damage; subjects with TLWM increases, most likely representing microdysgenesis; and subjects with both. This heterogeneity is hardly surprising as the group of MRI-negative patients will include patients with any pathology not detectable with current optimal MRI. Both hippocampal decreases and white matter increases in FMZ binding were detected in the group comparison despite subject heterogeneity. We therefore believe that both are common mechanisms in MRI-negative TLE.

Our decisions on which patients to include were based on the electroclinical diagnosis of TLE. Ictal video–EEG was not obtained in seven patients, but we do not believe that overemphasis should be placed on ictal EEG in this situation. A case in point is Patient 7 (Table 1), who would have been misclassified as having right TLE on the basis of four ictal surface EEG recordings but was correctly classified as having bitemporal foci on the basis of the remainder of the electroclinical data. FMZ–PET subsequently showed bilateral mesial temporal decreases in FMZ-Vd, and depth recordings confirmed the bilateral independent onset of seizures. In suspected bitemporal epilepsy, FMZ–PET may therefore help to confirm the bilateral origin (Ryvlin et al., 1998).

In our series, three patients (Patients 8, 17 and 18) have undergone modified anterior temporal lobe resections to date. Two (Patients 8 and 18) are currently seizure-free 26 and 4 months after the operation, respectively, and one (Patient 17) has had a single seizure within 2 weeks of starting fluoxetine therapy after 4 months without seizures, compared with six seizures per month before the operation. Despite the short follow-up in the last two cases, this suggests that the decrease in hippocampal FMZ-Vd detected by SPM indeed reflected the epileptic focus.

Two of the operated cases (Patients 8 and 17) had ipsilateral hippocampal decreases in FMZ-Vd accompanied by amygdala decreases in the case of Patient 17. For the patient operated in our centre (Patient 17), pathology was available and showed amygdala sclerosis as well as mild hippocampal end folium sclerosis, confirming that end folium sclerosis and amygdala sclerosis may be associated with decreases in FMZ-Vd, even if high-resolution MRI is unremarkable.

In two of the operated cases (Patients 17 and 18), sufficient amounts of TLWM were available to quantify TLWM neuronal density immunohistochemically with NeuN, a specific neuronal marker, as described recently (Thom et al., 2001). In Patient 17, no TLWM increases were detected on FMZ PET, and immunohistochemistry confirmed low neuronal densities (1010/mm³). In Patient 18, contralateral but not ipsilateral increases in TLWM FMZ binding were seen. Immunohistochemistry showed ipsilateral neuronal densities in the high normal range (2368/mm³). Ipsilateral high normal neuronal densities are compatible with the concept of microdysgenesis as a diffuse process, while the fact that they were not significantly elevated corroborates our PET finding.

Another patient (Patient 15) with a small area of significant FMZ-Vd decrease in the ipsilateral temporal neocortex and bilateral TLWM FMZ-Vd increases was operated upon and
found to have a malformative lesion similar to a Taylor-type focal cortical dysplasia (Taylor et al., 1971). Pathology showed abnormal white matter neurones, although the amount of resected white matter was not sufficient to quantify it. He remains seizure-free 25 months after the operation. This case confirms that focal cortical malformations may be associated with decreases in FMZ-Vd, even if MRI is unremarkable, and represents further evidence for the pathological equivalent of increases in FMZ-Vd detected in vivo. Furthermore, besides the confirmation that malformations may indeed be associated with increases in FMZ-Vd in white matter, in this single case, at least, bilateral white matter changes did not seem to affect the prognosis adversely after surgery in the limited duration of available follow-up.

This would be in keeping with an early study of temporal lobe surgery, before MRI diagnosis of hippocampal sclerosis in vivo became widely available (Hardiman et al., 1988). Fifty patients were treated with anterior temporal lobe resection, sparing the hippocampus and the amygdala. Severe neuronal ectopia was present in 42% of patients, and 28% showed neuronal clustering. Interestingly, both were predictive of good clinical outcome. The rate of seizure-free patients (52%) was lower than in contemporary series with removal of the hippocampus and amygdala. The study does, however, raise the intriguing possibility that, in some cases with microdysgenesis, resection of heterotopic white matter neurones may be sufficient to achieve freedom from seizures and suggests the possibility that seizures may arise from epileptogenic networks that involve white matter.

A caveat when considering patients with bilateral TLWM increases is reports of worse postoperative outcome after surgery in patients with intractable TLE (mostly found to have hippocampal sclerosis) when there were high numbers of white matter neurones (Kasper et al., 1999) and our own finding of a positive correlation between increased FMZ-Vd in the TLWM contralateral to the lobe resected for hippocampal sclerosis with outcome poorer than Engel class IA (Hammers et al., 2001a).

In contrast, Choi and colleagues found a better surgical outcome for hippocampal sclerosis patients with MRI-detectable TLWM changes which correlated with heterotopic neurone number (Choi et al., 1999). The groups were, however, defined through the MRI findings before white matter neurones were counted, and the fact that patients with more damage tend to have a better outcome (Jack et al., 1992) may have been a confounding factor. Finally, Thom and colleagues used immunohistochemistry and a rigorous quantitative approach with 3D cell counting methods in 31 patients with MRI-diagnosed and pathologically confirmed hippocampal sclerosis (Thom et al., 2001). The 17 out of 26 patients with sufficient follow-up who became seizure-free had significantly higher TLWM neuronal densities and other microdysgenetic features than the nine out of 26 who did not. As with all surgical studies, only tissue ipsilateral to the hippocampal sclerosis could be studied.

These findings need not be contradictory. It is conceivable that microdysgenesis may sometimes be due to generalized disturbances of neuronal migration or cell death. In these cases, heterotopic neurones are merely a marker of more widespread disturbance and consequently prognosis may be affected adversely (Hammers et al., 2001a). In other cases, however, when migration or cell death are disturbed only locally, surgical interruption of abnormal circuits involving heterotopic neurones (Chevassus-au-Louis et al., 1998) may be sufficient to achieve freedom from seizures, and in these cases microdysgenesis may indicate a better prognosis (Hardiman et al., 1988; Choi et al., 1999; Thom et al., 2001).

In summary, we have optimized the analysis of FMZ–PET scans by adapting SPM99 to the analysis of grey and white matter changes in TLE with normal high-resolution and quantitative MRI. We were able to increase the yield of both non-invasively detected abnormalities and surgically relevant abnormalities. Inclusion of the white matter in the analysis provides new and interesting insights into the pathophysiology of ‘MRI-negative’ TLE. FMZ–PET provides useful information in a significant proportion of potential surgical candidates.

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