Central benzodiazepine receptors in malformations of cortical development
A quantitative study

Alexander Hammers,1,2 Matthias J. Koepp,1,2 Mark P. Richardson,1,2 Claire Labbé,1 David J. Brooks,1 Vincent J. Cunningham1 and John S. Duncan1,2

1MRC Cyclotron Unit, Clinical Sciences Centre, Hammersmith Hospital and 2National Society for Epilepsy, Institute of Neurology, London, UK

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

Summary
We calculated [11C]flumazenil volume of distribution ([11C]FMZ-Vd) after correction for partial volume effect in 10 patients with malformations of cortical development (MCDs) and partial seizures, to quantify the GABA\(_A\)-central benzodiazepine receptor complex. Abnormal grey matter and adjacent or overlying cortex were outlined individually and added to an individualized anatomical template for correction for partial volume effect. Nine of 10 patients showed single or multiple increases or decreases in [11C]FMZ-Vd in or around MCDs. Two of three patients with band heterotopia showed multiple increases in the overlying cortex. In three of four patients with subependymal nodular heterotopia, nodules had lower [11C]FMZ-Vd than the overlying cortex, which was normal. Decreases in [11C]FMZ-Vd were found in two of three clefts and one of six adjacent regions in one schizencephalic patient; another had normal [11C]FMZ-Vd in the thickened cortex itself but increases in all adjacent regions. Binding was reduced within focal cortical dysplasia but increased in adjacent cortex. [11C]FMZ-Vd was normal within one patient’s polymicrogyric cortex but increased in one of six adjacent volumes of interest. The localization of abnormalities correlated with EEG and clinical data in cortical MCDs. Flumazenil binding was decreased in some MCDs with increased grey matter volume and increased in some adjacent or overlying areas of normal-appearing cortex, suggesting functional abnormalities beyond MRI-detectable structural changes.

Keywords: flumazenil; epilepsy; PET; malformations of cortical development

Abbreviations: BHT = band heterotopia; FMZ = flumazenil; [11C]FMZ-Vd = [11C]flumazenil volume of distribution; cBZR = central benzodiazepine receptor; CPS = complex partial seizures; FCD = focal cortical dysplasia; MCD = malformation of cortical development; PMG = polymicrogyria; SNH = subependymal nodular heterotopia; SPM = statistical parametric mapping

Introduction
Malformations of cortical development (MCDs) are due to disorders of neuronal and glial proliferation, abnormal neuronal migration or abnormal postmigrational cortical organization, and are present in 15–20% of adults with intractable partial seizures, some of whom are candidates for epilepsy surgery (Kuzniecky and Jackson, 1997). Surgical resection of areas of MCD in patients with drug-resistant epilepsy, however, results in only about 20–40% of patients becoming seizure-free (Cascino et al., 1993; Sisodiya, 2000) compared with 70% of patients with hippocampal sclerosis (Berkovic et al., 1995). A possible explanation is that the area of functional cortical abnormality may be greater than the structural abnormality shown by conventional MRI techniques (Sisodiya et al., 1995; Richardson et al., 1996, 1997).

Animal models have shown functional abnormalities in the cortex adjacent to MCDs, possibly as a result of the formation of aberrant thalamocortical connections subsequent to the presence of MCDs in the original projection area (Jacobs et al., 1999a, b). Abnormal adjacent cortex might explain in part the low surgical success rate in MCDs and the observation that lesionectomies tend to have a less good outcome than more extended resections (Raymond et al., 1995).
GABA is the principal inhibitory neurotransmitter in the brain, acting at the GABAA-receptor or benzodiazepine receptor complex, and plays an important role in the genesis of partial seizures. Flumazenil (FMZ) is a specific, reversibly bound, high-affinity neutral antagonist of cBZR (Olsen et al., 1990), and [11C]FMZ-PET provides a useful in vivo marker of GABAA-cBZR binding (Maziere et al., 1984).

The limited spatial resolution of PET results in partial volume effect that affects particularly the quantification of signals in structures smaller than twice the full width at half maximum resolution of the scanner used (Hoffman et al., 1979), such as the cortical ribbon or small heterotopic nodules, because of tissue averaging effects. Correction for partial volume effect is particularly important when structural abnormalities are present. Subtle changes in grey matter content in MCDs may not be detected on visual inspection of high-quality MRI (Kuzniecky et al., 1984; Desbiens et al., 1991). Without correction for partial volume effect, it is not possible to distinguish if an abnormality detected using PET represents a true functional abnormality due to a change in receptor density or affinity per neurone, a structural abnormality due to an increase or decrease in grey matter, or both of these together (Müller-Gartner et al., 1992; Rousset et al., 1993; Labbé et al., 1998).

Voxel-based methods of analysis (statistical parametric mapping, SPM) have been applied to [11C]FMZ-PET and MRI (Richardson et al., 1996, 1997) and have shown abnormalities of [11C]FMZ-Vd that were frequently more extensive than structural changes seen on MRI. These findings indicated that some of the PET abnormalities could be accounted for by abnormalities of cortical grey matter volume. The voxel-based SPM approach, however, does not give quantitative estimates of [11C]FMZ binding to cBZR and was not suited to the specific testing of hypotheses about normal-appearing cortex adjacent to or overlying MCDs.

The aims of the present study were (i) to determine if there were changes in [11C]FMZ-Vd after correction for partial volume effect, within areas of abnormal grey matter due to MCDs, within the overlying and adjacent areas of cortex that appeared structurally normal on high-resolution MRI and within distant cortical areas; and (ii) to correlate clinical and EEG abnormalities with PET findings.

Material and methods

Patients and controls

We studied 10 patients (six women) with partial seizures and MCDs that were diagnosed on high-resolution MRI, who 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 13 years (range 1–21 years), the median duration of epilepsy before the PET examination was 17 years (range 5–33 years) and the median age at PET examination was 30 years (range 18–47 years). The antiepileptic medication was carbamazepine (eight patients), phenytoin (two), lamotrigine (one), vigabatrin (one), sodium valproate (one) or ethosuximide (one) alone or in combination; six patients were taking 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.

Four patients had subependymal nodular heterotopia (SNH) (one had an additional parieto-occipital schizencephaly), three had band heterotopia (BHT), one had schizencephaly with three clefts (left frontal, right central and right temporoparietal), one had focal cortical dysplasia (FCD) in the temporal lobe and one had bilateral perisylvian polymicrogyria (PMG).

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, and the approvals of local ethics committees (Ethics Committee, Imperial College, Hammersmith Hospital and Joint Medical Ethics Committee of The Institute of Neurology and The National Hospital for Neurology and Neurosurgery) and the UK Administration of Radiation Substances Advisory Committee were obtained.

Non-quantitative voxel-based data have been reported previously for eight of the patients and for seven of the controls (Richardson et al., 1996, 1997).

Clinical details for all 10 patients are shown in Table 1. None of our patients has undergone epilepsy surgery.

PET technique

We used the same acquisition technique as described previously (Richardson et al., 1996, 1997). Briefly, PET scans were performed in 3D mode with the septa retracted, using a 953B Siemens/CTI PET camera with a reconstructed image resolution of about $8 \times 8 \times 4 \text{ mm}$ at full width half maximum for 31 simultaneously acquired planes (Spinks, 1992). Scans were performed with transaxial images obtained parallel to the plane defined by the anterior and posterior commissures and coronal images orthogonal to this. An eight-channel EEG was recorded during the PET studies to ensure that the scans were interictal. High-specific activity $[^{11}C]$FMZ tracer (Maziere et al., 1984) (370 MBq) was injected intravenously. Arterial blood was sampled continuously in order to determine a metabolite-corrected plasma input function. A dynamic 3D series, consisting of 20 frames over 90 min, was acquired for the brain volume. A convolution subtraction scatter correction was used (Bailey, 1992). The 20 time frames of the dynamic image were
<table>
<thead>
<tr>
<th>No.</th>
<th>MCD type</th>
<th>Age (years)/gender</th>
<th>Age of onset (years)</th>
<th>Syndrome type</th>
<th>EEG (inter-ictal)</th>
<th>Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bil EHT</td>
<td>3/M 2</td>
<td>2</td>
<td>CPS 2° gen</td>
<td>Ireg gen spikes</td>
<td>R FL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L FL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R FL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L OL</td>
</tr>
<tr>
<td>2</td>
<td>Bil EHT</td>
<td>30/M 12</td>
<td>12</td>
<td>SPS: motor R arm 2° gen</td>
<td>L hemi slow</td>
<td>R FL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L FL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R FL</td>
</tr>
<tr>
<td>3</td>
<td>Bil EHT</td>
<td>22/M 18</td>
<td>18</td>
<td>CPS Normal</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>Bil Schiz</td>
<td>34/M 1</td>
<td>1</td>
<td>SPS: motor L arm 2° gen</td>
<td>No def. abnormality</td>
<td>R cent cenl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R temp-par cenl</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>adj to L temp cenl</td>
</tr>
<tr>
<td>5</td>
<td>L post SNH</td>
<td>29/M 19</td>
<td>19</td>
<td>SPS: motor L arm 2° gen</td>
<td>Bil SW</td>
<td>L post SNH</td>
</tr>
<tr>
<td>6</td>
<td>SNH</td>
<td>47/M 21</td>
<td>21</td>
<td>CPS 2° gen</td>
<td>Bil temp loci</td>
<td>R ant SNH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R post SNH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L ant SNH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L post SNH</td>
</tr>
<tr>
<td>7</td>
<td>R par-occ SNH</td>
<td>26/M 19</td>
<td>19</td>
<td>SPS 2° gen</td>
<td>No def. abnormality</td>
<td>R par-occ SNH</td>
</tr>
<tr>
<td>8</td>
<td>Bil occ</td>
<td>20/F 2</td>
<td>2</td>
<td>CPS (visual) 2° gen</td>
<td>R post hemisph</td>
<td>adj to R par-occ cent</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ant</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>middle</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>post</td>
</tr>
<tr>
<td>9</td>
<td>R temp FCD</td>
<td>18/M 7</td>
<td>7</td>
<td>CPS 2° gen</td>
<td>R front-temp</td>
<td>R temp FCD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>adj to R temp FCD</td>
</tr>
<tr>
<td>10</td>
<td>Bil pect- anam</td>
<td>30/M 14</td>
<td>14</td>
<td>L focal motor</td>
<td>Tonic</td>
<td>R mid-temp slow</td>
</tr>
</tbody>
</table>

*2° gen. = secondarily generalized; adj. = adjacent; ant./post. = anterior/posterior; BHT = band heterotopia; bil. = bilateral; defin. = definite; F/M = female, male; FCD = focal cortical dysplasia; FL/PL/OL = frontal/parietal/occipital lobe; GM = grey matter; hemi. = hemisphere; MCD = malformation of cortical development; n.a. = not applicable; PMG = polymicrogyria; schiz. = schizencephaly; SNH = subependymal nodular heterotopia; SPS/CPS = simple/complex partial seizures; SW = spike-wave; RL = right/left; temp./par./occ. = temporal/parietal/occipital; VOI = volume of interest. Imaging findings: black = MCD itself (band heterotopia not shown); grey = cortex overlying the subcortical MCD or adjacent to the cortical MCD; red = increased [11C]FMZ-Vd binding compared with controls.*
realigned with one another by an automated least-squares technique to minimize any movement artefact during the scan (Friston et al., 1995). Parametric images of \(^{[11]C}\)FMZ-V\(_d\), reflecting binding to cBZR at the voxel level (Koepppe et al., 1991), were produced from the brain uptake and plasma input functions using spectral analysis (Cunningham and Jones, 1993).

**PET image analysis**

Analyze version 7.5 (Robb and Hanson, 1991) and MATLAB (Mathworks, Sherborn, Mass., USA) were used to perform image manipulation and measurements on Sun Ultra 10 workstations (Sun Microsystems, Mountain View, Calif., USA). The spatial transformations were based on software included in the SPM99 package (Wellcome Department of Cognitive Neurology, London, UK).

The aim was to quantify \(^{[11]C}\)FMZ binding after correction for partial volume effect in areas of abnormal grey matter due to MCDs, and in normal-appearing cortex overlying the subcortical MCDs or adjacent to the cortical MCDs. These areas were outlined manually on the MRI scans as follows. (i) Areas containing only thickened grey matter, representing the MCD itself, were defined first. (ii) In the cortical forms of MCD, adjacent volumes of interest, consisting of cortex which appeared normal on MRI, were then defined on transverse planes. Extrapolating from findings in animal models, we outlined ~1.5 cm of normal-appearing brain, comprising both grey matter and white matter, for later automatic segmentation. (iii) In the case of discontinuous grey matter abnormalities (Patients 8 and 10) (Table 1), more than two adjacent volumes of interest were defined. (iv) In the subcortical forms, the volumes of interest of overlying cortex were defined in the dependent migrational zone overlying the SNHs, by dropping a perpendicular from the SNH to the cortical surface and allowing for ~20° of lateral migration. These sections included grey and white matter. The subependymal heterotopion in Patient 5 and the two anteriorly located band-like subependymal heterotopia in Patient 6, however, were too small to be themselves delineated accurately on the MRI images. This did not prevent the delineation of the overlying cortex (Table 1). Because of the widespread nature of the BHT, all cortical volumes of interest of the convexity were considered overlying cortex.

We used an MRI scan of a single brain obtained at high resolution at the Montreal Neurological Institute (Holmes et al., 1998), as delivered with the SPM99 package, and an interactive algorithm to create a brain template consisting of 29 volumes of interest (Hammers et al., 2000). This was first transformed into the individual patient’s MRI space. The individually outlined additional volumes of interest (i.e., MCDs and overlying or adjacent cortex) were added to the template. The high-resolution volume-acquisition MRI scans were then segmented automatically into probability images of grey matter, white matter and CSF (Hartigan, 1975), excepting the individually outlined areas of grey matter within the MCDs. The grey matter, white matter and CSF images and the volumes of interest were first coregistered with the PET data (Woods et al., 1993) and then blurred to the same spatial resolution as PET by convolving each segmented probabilistic MRI with the 3D point spread function of the PET scanner. A least-squares weighted fit of these blurred images to the observed PET images was then calculated (Labbé et al., 1998). This allowed estimates of partial volume effect within the multiple volumes of interest of homogeneous tracer activity to be obtained. To obtain a control range for \(^{[11]C}\)FMZ-V\(_d\) in equivalent cerebral areas to the MCD and surrounding cortex, the individualized template for each patient was spatially normalized to the MRI scans of all 21 controls, and \(^{[11]C}\)FMZ-V\(_d\) values corrected for partial volume effect were obtained for the corresponding grey matter areas in healthy volunteers (Fig. 1).

**Statistical analysis**

We defined the normal range for the absolute \(^{[11]C}\)FMZ-V\(_d\) values after correction for partial volume effect as 2.5 SDs from the normal control mean for the areas corresponding to individually outlined MCDs and adjacent/overlying volumes of interest, and for all other standard volumes of interest as 3 SD from the mean. These strict thresholds were chosen to allow for the large number of comparisons. The lower threshold was used for MCDs and surrounding cortex as we had specific hypotheses that these would be abnormal. The rationale behind the choice is a calculation of the numbers of false positives expected for the number of comparisons (1.56 for an average of six regions with specific hypotheses, a threshold of ±2.5 SD and 21 controls; and a very similar value, 1.58, for 29 regions without specific hypotheses, a threshold of ±3 SD and 21 controls). There was no significant difference between the corrected \(^{[11]C}\)FMZ-V\(_d\) values obtained for the right and left sides of the standard anatomical volumes of interest in controls, so these values were considered together. Statistical analysis was performed using Pearson’s and Spearman’s correlation coefficients (r, r\(_s\)), Student’s t test, Wilcoxon’s rank-sum test, the \(\chi^2\) test and the Kolmogorov–Smirnov test where indicated.

**Results**

**Absolute \(^{[11]C}\)FMZ-V\(_d\) with correction for partial volume effect**

**Controls**

In each set of 21 controls individualized for each of the 10 patients, the values obtained for the volumes of interest were normally distributed, including the corrected \(^{[11]C}\)FMZ-V\(_d\) values for regions derived from the manually outlined MCDs and overlying/adjacent cortex. In nine of the 10 control sets, one value for the left insula was >3 SD above the mean. In one control set, the value for the left cingulate gyrus fell >3
The standard anatomical template was first normalized into the patient’s MRI space. The lesion and adjacent or overlying cortex were outlined on the patient’s MRI and combined with the normalized template to create an individualized template for each patient. This was subsequently transformed to all 21 controls and $[^{11}\text{C}]$FMZ-$V_d$ values corrected for partial volume effect were calculated for all of them, thus giving a normal range for each volume of interest for each patient.
SD above the mean. This is not unexpected given the ~600 control values obtained for comparison with each patient.

**Patients**

**MCDs and adjacent/overlying volumes of interest.** (i) BHT: of the three patients with BHT, one (Patient 3) had a normal FMZ-PET scan and two (Patients 1 and 2) showed multiple areas of increased \([^{11}C]FMZ-V_d\) in the overlying cortex (Table 1). (ii) SNH: of the four patients with SNH (Patients 5–8), three (Patients 5–7) showed significantly less flumazenil binding in the heterotopic nodules than in the overlying cortex; the overlying cortex itself was normal in all four (Table 1). (iii) Schizencephalies: \([^{11}C]FMZ-V_d\) corrected for partial volume effect was lower in two of the three clefts in one schizencephalic patient (Patient 4) than in equivalent normal grey matter, with an additional decrease in one out of six adjacent regions; the other patient (Patient 8) had normal \([^{11}C]FMZ-V_d\) in the thickened cortex itself but significant increases in all adjacent regions (Table 1). (iv) FCD: binding was increased in the cortex immediately adjacent to the FCD (Patient 9), which itself showed significantly reduced partial volume effect-corrected \([^{11}C]FMZ-V_d\) (Table 1). (v) PMG: after correction for partial volume effect, the patient with bilateral PMG (Patient 10) showed normal \([^{11}C]FMZ-V_d\) within the polymicrogyric cortex but a significant increase in one of the six adjacent volumes of interest (Table 1).

**Standard anatomical volumes of interest.** None of the standard anatomical volumes of interest lay outside the individual control ranges for any of the patients.

**Correlations with EEG and clinical data**

The localization of maximum abnormalities (both decreases and increases) correlated with EEG and clinical data in both schizencephalic patients, in the patient with FCD and in the patient with PMG, i.e. in the cortical forms of MCD.

**Schizencephalies**

Patient 4 suffered from partial motor seizures affecting the left arm, and secondarily generalized seizures. Correspondingly, the maximum decreases in \([^{11}C]FMZ-V_d\) were found in the thickened cortex in the right central cleft (−41%), with additional significant decreases in the thickened cortex in the right temporoparietal cleft and in one of two volumes of interest adjacent to the left frontal cleft (−24 and −29%, respectively).

Patient 8 had visual complex partial seizures (CPS) and secondarily generalized seizures. Her bilateral occipital SNH showed normal binding compared with the overlying cortex, as did the thickened cortex within her right parieto-occipital schizencephalic cleft. In all three neighbouring volumes of interest, however, \([^{11}C]FMZ-V_d\) was significantly increased (by 38, 41 and 69%, respectively). Interictal EEG abnormalities were localized in the right posterior hemisphere.

**FCD**

Patient 9 had temporal lobe type CPS with secondary generalization. \([^{11}C]FMZ-V_d\) showed a significant decrease within the right temporal FCD (−51%), with a significant increase (+35%) in one of the two neighbouring cortical areas in the right temporal lobe. Interictal EEG abnormalities were localized in the right frontotemporal region.

**Bilateral perisylvian PMG**

Patient 10 had partial motor seizures affecting the left side of his body as well as tonic seizures. After correction for partial volume effect, the areas of polymicrogyric cortex were shown to have normal \([^{11}C]FMZ-V_d\). One of the volumes of interest in the adjacent cortex in the central region on the right that appeared normal on MRI showed a significant increase in \([^{11}C]FMZ-V_d\) (−23%). Interictal EEG changes were localized in the right midtemporal region.

There were no significant correlations of \([^{11}C]FMZ-PET\) and clinical and EEG data in the patients with subcortical forms of MCD (BHT and SNH) (Table 1).

**Discussion**

This is the first study to apply a quantitative MRI-based multiple volume of interest approach with correction for partial volume effect to parametric images of cerebral \([^{11}C]FMZ\) binding of patients with MCDs. Abnormalities of partial volume effect-corrected \([^{11}C]FMZ-V_d\) were detected in nine out of 10 patients.

The main novel finding was a general pattern of decreased \([^{11}C]FMZ\) binding in areas of increased grey matter volume, e.g. heterotopic nodules or PMG, and increased \([^{11}C]FMZ\) binding in adjacent or overlying areas of normal cortex across the various subtypes of MCD, compared with normal neocortex. Moreover, the localization of abnormal \([^{11}C]FMZ\) binding correlated with EEG and clinical data in the cortical forms of MCD (schizencephaly, FCD and PMG). Using this method, absolutely quantified PET data obtained in vivo are in good accordance with experimental and in vitro data.

**Methodological considerations**

Partial volume effect is a consequence of the limited spatial resolution of PET. Correction for partial volume effect is necessary in order to quantify changes accurately and distinguish between functional changes that merely reflect structural changes (atrophy or increased grey matter content) and functional changes per se, e.g. changes in binding to receptors on neurones (Frost et al., 1995; Rousset et al., 1995; Labbé et al., 1996; Koepp et al., 1998).
In the present study, we used a region template of multiple cortical volumes of interest defined in standard stereotactic space, and subsequently transformed into each subject’s MRI and PET space. The use of such a template, its automated coregistration and subsequent correction for partial volume effect provided an entirely objective and observer-independent method for defining multiple neocortical volumes of interest and then quantifying $^{11}$C FMZ-Vd in them (Labbé et al., 1998; Koeppe et al., 2000). This approach works satisfactorily for the neocortex if there is no structural abnormality (Koeppe et al., 2000; Hammers et al., 2001), but the presence of MCDs of different shapes and sizes in each individual patient necessitated that the structures of particular interest, in this case the areas of abnormal grey matter due to MCDs and the adjacent/overlying cortex, were outlined individually by hand. By applying this individualized template, specific for one patient, to all 21 controls for each patient, we were able to define normal ranges of equivalent areas of the brain for each of the individual volumes of interest. However, this method is computationally very demanding, as each patient was evaluated individually against all 21 controls.

The method used depends on an automatic segmentation algorithm for the areas outside the MCD itself, whereas within the MCD abnormal grey matter was outlined by hand and not segmented. Inspection of the segmented grey matter images of the volumes of interest outside the MCD showed that the segmentation was visually acceptable. Although a small error due to misclassification of some voxels cannot be entirely ruled out, this is very unlikely to have influenced our results as probability images were used, i.e. the probability of a given voxel belonging to the tissue class of grey matter was used in the calculations rather than simple binary decisions (Hartigan, 1975; Ashburner and Friston, 1997). Even changed characteristics of the grey matter–white matter interface should be accommodated by this method.

Using voxel-based analyses (SPM) applied to parametric images of $^{11}$C FMZ binding in MCDs, we demonstrated previously areas of abnormal $^{11}$C FMZ-Vd in 10 out of 12 patients (Richardson et al., 1996). These areas frequently extended beyond the lesions visible on MRI and consisted of increased as well as decreased $^{11}$C FMZ-Vd. With this approach, however, some of the changes in $^{11}$C FMZ binding could simply have reflected changed grey matter content, i.e. merely structural changes. In a second step, we combined the information from statistical parametric maps of MCD patients’ $^{11}$C FMZ-Vd images with maps of these patients’ structural MRIs (Richardson et al., 1997). This approach showed areas of disproportionate abnormal $^{11}$C FMZ binding in six out of 10 patients, including areas which appeared functionally normal on examination of PET data alone. The method could not, however, quantitate absolute binding abnormalities, and areas of SNH were neglected by this study, which was confined to the neocortical shell of grey matter.

Of the seven patients who were included in both our current and the previous study (Richardson et al., 1997) (Patients 1, 2 and 4–8), findings of abnormal $^{11}$C FMZ-Vd in the neocortical shell were very similar for three (Patients 1, 4 and 5). Additional areas of abnormalities were found in two (Patients 2 and 8), which were most likely attributable to our working with absolutely quantified values in Patient 2 and to correction for partial volume effect in Patient 8. Relatively small abnormalities seen with the SPM-based method in areas remote from the MCDs could not be replicated in two patients (Patients 6 and 7), most likely because the small areas of abnormalities that were detected were averaged in the larger volumes of interest employed in the present study. In both these cases, abnormalities were found within or adjacent to the MCDs instead in the present study.

None of our patients were taking benzodiazepines prior to the PET studies. One patient each, however, was taking vigabatrin and sodium valproate. Vigabatrin inhibits the GABA transaminase irreversibly and therefore elevates brain GABA concentrations up to threefold. This does not, however, lead to any alteration of cBZR binding (Verhoeff et al., 1999). One of the mechanisms of action of sodium valproate is via GABAergic properties. Again, this does not alter cBZR binding in man in vivo (Koeppe et al., 1997b). Moreover, our two patients on vigabatrin and sodium valproate did not show global changes in $^{11}$C FMZ binding, as would be expected if the drugs acted on the benzodiazepine binding site or led to up- or downregulation of cBZR receptors (Savic et al., 1991). The perilesional increases in Patient 8 (on sodium valproate) and in Patient 10 (on vigabatrin) are therefore unlikely to be explained by their medication.

**Clinical and neurobiological considerations**

The exact pathological and pathophysiological mechanisms underlying epileptogenesis in MCDs are still under study. While MCDs can be intrinsically epileptogenic (Mattia et al., 1995; Palmini et al., 1995; Sisodiya et al., 1999), the generally poor results after epilepsy surgery (Engel, 1993), quantitative MRI findings (Sisodiya et al., 1995) and PET studies (Richardson et al., 1996, 1997; Ryvlin et al., 1998; Van Bogaert et al., 1998), as well as animal models (Jacobs et al., 1999b), indicate that structural and functional abnormalities are more widespread than the structural lesion visualized by MRI.

Epileptogenic foci have generally been reported to exhibit decreased $^{11}$C FMZ binding (Savic et al., 1988, 1993, 1995; Henry et al., 1993; Szelies et al., 1996; Koeppe et al., 1997a, 2000; Richardson et al., 1998; Ryvlin et al., 1998; Muzik et al., 2000), so the finding of cortical areas of decreased $^{11}$C FMZ binding may indicate the localization of the focus. Some studies, however, have found localized increases in the number or affinity of cBZR–GABA$_A$ receptors (Hand et al., 1997; Brooks-Kayal et al., 1998), and it is also possible that areas of increased $^{11}$C FMZ-Vd mark the epileptogenic zone in some forms of focal epilepsy.
Subcortical forms of MCD (BHT, SNH)

We did not detect any cortical decreases in $[^{11}C]$FMZ binding in the patients with heterotopia (Patients 1–3 and 5–7), i.e. subcortical forms of MCD. Two of the three patients with band heterotopia (Patients 1–3) showed widespread bilateral significant increases in $[^{11}C]$FMZ-Vd in volumes of interest overlying the band heterotopia, in keeping with the generalized nature of these MCDs. In contrast to the band heterotopia, the subependymal nodular heterotopia (Patients 5–7) were big enough to be outlined individually. In each case, $[^{11}C]$FMZ-Vd (corrected for partial volume effect) within the nodules was significantly less than the normal values for the overlying cortex, but the overlying cortex appeared normal.

This result is in keeping with the immunocytochemical finding of less morphologically complex GABAergic interneurones within nodules in autopsy and surgical material. These interneurones showed less neuropeptide Y binding, indicative of fewer synapses being present and implying that these neurones were less mature than their counterparts in the overlying cortex. This conclusion is reinforced by a study using proton magnetic resonance spectroscopy (Marsh et al., 1996). The decrease in inhibitory function in these nodules as shown by this study, together with the fact that there are projections out of the nodules (Hannan et al., 1999), may explain the high epileptogenicity of these lesions.

An in vitro study (Hannan et al., 1999) described clusters of abnormal GABAergic interneurones in the cortex overlying subcortical heterotopic nodules, but did not report whether there was an associated increase in FMZ binding (see below).

An interesting observation is that, within the group of three patients with BHT, more widespread $[^{11}C]$FMZ-Vd abnormalities in the cortex correlated with more severe epilepsy (as defined by younger age at onset, more widespread EEG changes, more medication and more frequent seizures).

Cortical forms of MCD (schizencephalies, FCD, PMG)

Some of the patients with cortical forms of MCD (Patients 4 and 8–10) showed normal $[^{11}C]$FMZ binding, corrected for partial volume effect, within the thickened cortex, indicating normal cBZAR binding per volume of grey matter (Patients 8 and 10). Two others showed decreases in the thickened cortex (Patients 4 and 9). This is in keeping with histopathological evidence for low numbers of calcium-binding protein-immunopositive GABAergic interneurones within abnormal cortex in FCD in surgical specimens (Spreatico et al., 1998) and with significantly reduced binding to GABA_A receptors in dysplastic tissue and, albeit to a lesser degree, in the exofocal cortex in an animal model (Zilles et al., 1998), as in our Patient 4.

In the present study, the surrounding cortex, which appeared normal on high-resolution MRI, showed significant increases in $[^{11}C]$FMZ-Vd in three of these four patients, implying a pattern of decreased inhibition inside the MCD with increased inhibition in the adjacent areas. From the freeze microgyrus model, there is evidence of enhanced inhibitory function in perilesional areas (Prince and Jacobs, 1998) and of abnormalities outside the actual structural lesion, as well as epileptogenesis in the paramicrogyral zone (Jacobs et al., 1999a). This MCD also leads to widespread disruption of cortical organization, probably due to aberrant thalamocortical projections (Jacobs et al., 1999c). Human data are scarce, partly because of the small numbers of patients with MCDs who undergo surgery. A case report has shown large numbers of neurones in the white matter underlying the normal cortex close to PMG (Battaglia et al., 1996), which could explain our observation of a perilesional increase in our case with PMG.

The localization of the peak binding abnormalities was in keeping with EEG and clinical data in all four patients with cortical MCDs, which provides additional lateralizing information in those patients who had bilateral MCDs on MRI (Patients 4, 8 and 10). This information may prove useful in those patients with diffuse or bilateral MCDs for whom surgery is being considered (Raymond et al., 1995). A caveat is the unavailability of intracranial recordings in our study, as the interictal scalp EEG data may be equivocal (Raymond et al., 1995).

There are several possible explanations for increased $[^{11}C]$FMZ binding in the cortex adjacent to or overlying MCDs. These include an increase in the number of available receptors per neurone, and increased neuronal density or an increased number of ectopic neurones bearing cBZAR, as, for example, in microdysgenesis. It is conceivable that the cortex in the immediate vicinity of visible lesions may exhibit microdysgenesis that is not visible on high-resolution MRI (Desbiens et al., 1993) or detected by the MRI segmentation program. This would be in keeping with our recent finding of a correlation of neuronal density in the resected white matter of the temporal lobe, i.e. microdysgenetic changes, with $[^{11}C]$FMZ binding in patients with unilateral hippocampal sclerosis (Hammers et al., 2001). It is unlikely that increased $[^{11}C]$FMZ binding represents an adaptive upregulative response to epileptic activity, as increases in $[^{11}C]$FMZ binding were not seen in patients with acquired lesions that underlay epileptic activity (Richardson et al., 1998).

Our method does not distinguish between changes in the number of available binding sites ($B_{max}$) and changes in receptor affinity ($K_d$). Changes in both parameters have been described (Nagy et al., 1999). In this paper, spiking neocortex was examined in a wide variety of pathologies and compared with perilesional cortex and tissue from patients with severe head trauma. In a more homogeneous group of patients, all with hippocampal sclerosis and compared with autopsy controls, however, we found decreases in $B_{max}$ over and above neuronal loss in the CA1 region and increases in affinity, i.e. decreases in $K_d$ in some regions (Hand et al., 1997). Recently, postsynaptic increases in the number of GABA_A receptors underlying inhibitory potentiation in the
kindling model have been described (Nusser et al., 1998). As not all GABA<sub>A</sub> receptors bind benzodiazepines, increases in GABA binding will only be paralleled by increased [<sup>11</sup>C]FMZ binding if more GABA<sub>A</sub>-cBZR receptors are present. Using the pilocarpine model, pre- and postsynaptic changes in GABA transmission have been found (Brooks-Kayal et al., 1998) that involve changes in the β subunit composition of GABA<sub>A</sub> receptors, which may mediate affinity. Thus, increased density or affinity of available receptors per neurone, either on abnormal nerve cells or as a response to the abnormal circuitry in MCDs, may explain the observed increases in [<sup>11</sup>C]FMZ binding. Despite few patients with MCDs being good surgical candidates and the resulting scarcity of tissue available, correlative in vitro quantitative neuropathological and autoradiographic studies are in hand to address these questions.

Acknowledgements
We wish to thank our colleagues at the MRC Cyclotron Unit (especially Andrew Blyth, Matthew Brett, David Griffith, Joanne Holmes, Hope McDewitt, Ralph Myers and Leonhard Schnorr) for help in the acquisition and analysis of PET data and Iris Köth for graphical illustration of our results. We also wish to thank our clinical colleagues for referring patients and our colleagues at the National Society for Epilepsy for performing and transferring the MRI scans. This work was supported by the Deutsche Forschungsgemeinschaft (HA 3013/1–1), Action Research, the National Society for Epilepsy and the Medical Research Council.

References


