Cortical grey matter and benzodiazepine receptors in malformations of cortical development
A voxel-based comparison of structural and functional imaging data

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Summary
Using [11C]flumazenil-PET and statistical parametric mapping (SPM), we have shown recently that regions of increased and decreased benzodiazepine receptor density may be seen in patients with localization-related epilepsy due to malformations of cortical development. These abnormalities were seen both within and beyond lesions visually apparent on high-resolution MRI. We have also shown, using an interactive anatomical segmentation technique and volume-of-interest measurements, that subtle and unsuspected abnormalities of cortical grey matter volume were found in the same group of patients on high-resolution MRI, beyond the lesions visually apparent. In 10 patients with localization-related epilepsy and malformations of cortical development, we have now applied the automated and objective technique of SPM to the analysis of high-resolution structural MRI. Each individual patient was compared with 16 normal control subjects. We have then simultaneously compared the structural and functional data obtained for each individual patient with normal control high-resolution MRI and [11C]flumazenil-PET images using a novel technique. This comparison allowed the detection of functional abnormalities that were not accounted for by either visible or unsuspected structural abnormalities, in an automated and statistically rigorous manner. Five patients had abnormalities of cortical grey matter volume detected using SPM; only these five patients had been found abnormal using the previous volume-of-interest technique. Six of the 10 patients showed regions of cerebral cortex with disproportionate flumazenil binding compared with local grey matter volume. This included regions not found to have abnormal flumazenil binding on analysis of the PET data alone. Furthermore, regions found to have abnormal binding on examination of the PET data alone were, in some instances, shown to be accounted for by abnormalities of cortical grey matter volume. We conclude that the analysis of ligand PET data should always include a comparison with structural MRI; such comparisons are greatly facilitated by the novel approach described.

Keywords: statistical parametric mapping; PET; MRI; cortical dysgenesis; epilepsy

Abbreviations: ANCOVA = analysis of covariance; BZR–GABAA = benzodiazepine/γ-aminobutyric acid type A receptor; FMZVD = [11C]flumazenil volume of distribution; MRIGM = MRI-defined cortical grey matter image; SPM = statistical parametric mapping

Introduction
Ligand PET studies have been used successfully to investigate many neurological diseases including epilepsy (Savic et al., 1988; Mayberg et al., 1991; Kumlien et al., 1992; Theodore et al., 1992; Prevett et al., 1994; Richardson et al., 1996b), Parkinson’s disease (Brooks, 1993), Huntington’s disease (Holthoff et al., 1993; Turjanski et al., 1995; Weeks et al., 1996), Alzheimer’s disease (Labbe et al., 1996) and others. Conventionally, ligand parameters were measured in a region-
of-interest in a patient, or group of patients, and these data were compared with the homologous region in a control group. Although automated region identification has been proposed using a variety of different methods (Evans et al., 1991; Greitz et al., 1991), region placement generally relies on an observer’s expertise, is subject to observer bias and is limited in terms of the regions of brain evaluated. A further difficulty with regions-of-interest results from systematic differences between the anatomy of patient and control groups. In a degenerative disease such as Huntington’s disease, for example, the reduction of [11 C]flumazenil binding detected in the striatum may be a consequence of volume loss in this structure leading to partial volume effects. The apparently low ligand binding seen in an atrophied structure may actually be normal in terms of ligand binding per unit volume; indeed, partial volume effects leading to an apparent decrease in ligand binding could conceivably mask a real increase in binding per unit volume. Hence it has been recognized that methods of partial volume correction are necessary for a full characterization of PET data (Muller Gartner et al., 1992; Rouset et al., 1993; Frost et al., 1995; Rouset et al., 1995; Labbe et al., 1996). Those methods share in common the use of high-resolution MRI to define cerebral structure and its coregistration with the PET data.

In order to eschew the restrictions imposed by region-of-interest methods, particularly the subjectivity of region placement and the necessary limitation of the analysis to the selected regions, we recently undertook an analysis of [11 C]flumazenil-PET in patients with epilepsy using a voxel-based approach, statistical parametric mapping (SPM) (Richardson et al., 1996b). This study, and others (Koepp et al., 1996; Richardson et al., 1996a), demonstrated that SPM successfully detects not only significant differences in ligand binding in expected regions in the patient group compared with normal control subjects, but can also detect significant abnormalities in brain regions, that are not predicted in advance, with high specificity.

SPM has also been applied to the voxel-based analysis of structural MRI data (Wright et al., 1995). The next logical step is to attempt a voxel-based comparison of functional imaging and structural imaging in the same patient. PET data from a patient or patients can be compared with PET data from normal control subjects and the MRI from a patient or patients can be compared with the MRI of normal control subjects. The differences between the patients and control subjects within each of the two modalities can then be compared, both in terms of the anatomical position and relative magnitude, and the issue of partial volume correction of the PET data is bypassed. This allows the identification of apparent functional abnormalities in ligand binding that are actually simply proportional to a change in the volume of a grey matter structure at that point (that are not generally interesting) and, conversely, the identification of functional changes that are not related to, or not accounted for by, any structural change (and hence interesting). This represents a Group × Modality interaction.

As an example of this approach we have compared the structural and functional imaging data from patients with partial seizures due to malformations of cortical development. The data were derived from two published studies of the same group of patients: a study of regional cerebral volume measured on MRI in patients with partial seizures due to malformations of cortical development and in a normal control group (Sisodiya et al., 1995), and a study of cerebral [11 C]flumazenil binding to the benzodiazepine/γ-aminobutyric acid type A receptor complex (BZR-GABA_A) in patients with partial seizures due to malformations of cortical development and in a normal control group (Richardson et al., 1996b). The region-based data derived from the MRI study can be used as a validation of the voxel-based approach to MRI morphometry. We describe a method that is based entirely on existing tools in the current release of the SPM software at the time of writing (SPM95). This method not only contributes to our understanding of the specific issue of alterations of benzodiazepine receptor density in malformations of cortical development, but may also be applied generally to compare structural and functional data in the whole range of brain disorders.

Method

Subjects

We studied 10 patients who were recruited from the epilepsy clinics of the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK. All have previously been reported in two previous studies, one of MRI volumetry in malformations of cortical development associated with localization-related epilepsy (Sisodiya et al., 1995) and one of benzodiazepine receptor density in this disorder (Richardson et al., 1996b). The data from these studies were used. Patients were excluded from the PET studies if they were taking drugs which were thought to interact with the BZR-GABA_A complex (benzodiazepines, barbiturates or vigabatrin). Twenty-six normal subjects of similar age were studied in the PET investigation. These subjects had no evidence of neurological disorder and were on no medication. A separate group of 32 normal subjects were included in the MRI study. Sixteen normal control subjects were selected from each study control group in order to obtain the best possible age and sex match between the two control groups. Written informed consent was obtained from all subjects according to the declaration of Helsinki. Approval for the study was obtained from the local ethical committees at both the National Hospital for Neurology and Neurosurgery and the Hammersmith Hospital, and from the UK Administration of Radioactive Substances Advisory Committee.

Scanning protocol: PET

Scans were performed with subjects in the alert resting state using an ECAT-953B PET scanner (CTI/Siemens,
Knoxville, Tenn., USA) with performance characteristics as described previously (Spinks et al., 1992). Data were acquired in 3D mode, which results in an improvement in sensitivity by a factor of 6.4 compared with 2D acquisition (Bailey, 1992). Dual energy window scatter correction was employed in reconstructions to produce images with a resolution of $4.8 \times 4.8 \times 5.2$ mm. Images containing 31 contiguous slices were produced with voxel dimensions $2.09 \times 2.09 \times 3.43$ mm. The subject’s head was positioned with the glabella-inion line parallel to the detector rings, so that the transaxial plane was parallel to the intercommissural line. During scanning, the head was rested in an individualized head mould, and continuous direct observation maintained to minimize movement. An eight-channel EEG was recorded throughout to ensure that the observation maintained to minimize movement artefact (Friston et al., 1990, 1994, 1995). Data were analysed on a Sun SPARC classic workstation (Sun Microsystems, Mountain View, Calif., USA) using Analyze version 7.0 (Mayo Foundation) (Robb and Hanson, 1990), MATLAB (The MathWorks, Natick, Mass., USA) and SPM (Wellcome Dept of Cognitive Neurology, Institute of Neurology, London) (Friston et al., 1994, 1995a, b).

Data analysis
Data were analysed on a Sun SPARC classic workstation (Sun Microsystems, Mountain View, Calif., USA) using Analyze version 7.0 (Mayo Foundation) (Robb and Hanson, 1990), MATLAB (The MathWorks, Natick, Mass., USA) and SPM (Wellcome Dept of Cognitive Neurology, Institute of Neurology, London) (Friston et al., 1994, 1995a, b).

Spatial normalization of PET and MRI
All of the images were transformed into a standard 3D space. This normalizing spatial transformation matches each image to a reference or template image that already conforms to the standard anatomical space, without requiring user-defined landmarks. The procedure involves linear and quadratic 3D transformation followed by a 2D nonlinear matching, using a set of smooth basis functions that allow for normalization at a finer anatomical resolution (Friston et al., 1995a). Because the normal data for MRIGM and FMZVD were from different individuals, two separate but anatomically identical images were required as templates: one for the MRIGM images and one for FMZVD images. To ensure these templates were in exact register, one normal MRI was chosen. This image was segmented, as described above, to produce an MRIGM image and a second image with the thalami, basal ganglia and cerebellum included. Both of these images were flipped in the transverse plane and then

### Production of parametric maps of $[^{11}C]f$luromazenil binding
Radioactivity in arterial blood was assayed continuously online and intermittent blood samples were taken for calibration purposes, and for assay of radiolabelled metabolites in plasma (Luthra et al., 1993), to produce a metabolite-corrected input function. The 20 frames of the dynamic image were realigned to minimize movement artefact (Friston et al., 1995a). Voxel-by-voxel parametric images of $[^{11}C]f$luromazenil volume of distribution (FMZVD) were produced using the technique of spectral analysis (Cunningham and Jones, 1993). In the case of flumazenil, which has minimal non-specific binding, volume of distribution is closely correlated with receptor density (Koepe et al., 1991).

### Scanning protocol: MRI
MRI was performed using a 1.5-T GE Signa (GE, Milwaukee, Wis., USA). A coronal spoiled gradient recalled sequence (SPGR) was undertaken for morphometric analysis (TE 5 ms, TR 35 ms, flip angle 35°, acquisition matrix 256 $\times$ 128, 1 NEX, field of view 24 cm, producing 124 contiguous slices 1.5 mm thick).

### Segmentation of MRI and MRI volumetry
MRI images were transferred to an independent imaging analysis workstation (Allegro, ISG Technologies, Toronto, Canada). This permitted segmentation, division into regions-of-interest and subsequent volume measurements, as described previously (Sisodiya et al., 1995). In brief, the segmentation was achieved by a combination of selection of a pixel-intensity threshold with subsequent ‘region growing’ from a seed placed by the operator, then manual editing to separate other unwanted structures from the region-of-interest. A binarized image of the entire MRI-defined cortical grey matter from both cerebral hemispheres (MRIGM) was produced for each subject and used for analysis with SPM. Cortical grey matter and subcortical matter volumes-of-interest from each hemisphere were divided into 10 coronal regions-of-interest, each spanning one tenth of the total anterior–posterior extent of the hemisphere. The volume of each grey matter and subcortical block was measured and normalized for total intracerebral volume as described previously (Sisodiya et al., 1995). In this previous study a high degree of intra- and inter-observer reliability was established for the volume measurements. For each coronal volume-of-interest, measurements were made of the grey matter volume for each hemisphere, the subcortical matter volume for each hemisphere, the ratio of grey matter volumes from opposite hemispheres, the ratio of subcortical matter volumes from opposite hemispheres, and the ratio of grey matter volume and subcortical matter volume for each hemisphere. Thus, a total of 80 measures was produced for each brain. Our previous study showed no normal control subject had more than one abnormal measure; abnormality was defined as the presence of more than one abnormal measure.

### SPM of MRI and PET in epilepsy

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averaged with its unflipped counterpart to produce two symmetrical images. Finally, both images were smoothed with an 8-mm isotropic Gaussian kernel, to produce two anatomically identical images, one consisting of only cerebral cortex (the MRIGM template) and the other with cortex and subcortical structures (the FMZVD template).

**SPM**

Statistical parametric maps are statistical processes that are used to characterize regionally specific effects in imaging data. Effects were estimated according to the general linear model at each and every voxel (Friston et al., 1995b). To test hypotheses about regionally specific effects the estimates were compared using linear compounds or contrasts. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the $t$ statistic $SPM_{t}$.

For the analysis of each individual modality in isolation, the $SPM_{t,i}$ maps were transformed to the unit normal distribution ($SPM_{Z}$) and thresholded at $P < 0.001$. To correct for the multiple non-independent comparisons inherent in this analysis, the resulting foci were then characterized in terms of their spatial extent ($k$) (Friston et al., 1994). This characterization is in terms of the probability that a region of the observed number (or bigger) of voxels could have occurred by chance [$P(n_{max} > k)$] over the entire volume analysed (i.e. a corrected $P$-value). The corrected $P$-value chosen was $P < 0.05$.

**Analysis of structural data using SPM**

Each individual patient’s MRIGM image was compared with the normal group as described previously (Richardson et al., 1996b). All images were smoothed with a $13 \times 13 \times 9$ mm Gaussian kernel, resulting in an effective smoothness identical to FMZVD images smoothed at $10 \times 10 \times 6$ mm. The smoothed MRIGM images can be regarded as images in which voxel intensity reflects the local grey matter volume at a spatial resolution identical to that of the PET studies. Global mean voxel value was included as a confounding covariate and this analysis can therefore be regarded as an analysis of covariance (ANCOVA) (Friston et al., 1990). The occurrence and position of significant regional differences between the patients and the normal control subjects were compared with the results from the MRI volumetric analysis described above (Sisodiya et al., 1995), as a validation of the use of SPM in this context.

**Analysis of $^{11}$C]flumazenil-PET data**

Each individual patient’s FMZVD image was compared with the normal group as described previously (Richardson et al., 1996b). Images were smoothed with a $10 \times 10 \times 6$ mm Gaussian kernel. Global mean voxel value was included as a confounding covariate and this analysis can therefore be regarded as an ANCOVA (Friston et al., 1990). The occurrence and position of significant regional differences between the patients and the normal control subjects were noted.

**Comparison of MRI and PET images**

Having spatially normalized the images, the comparison of MRI and PET was greatly facilitated. The transformed, smoothed images were maps of local grey matter volume and local FMZVD at the same resolution. The question addressed at each voxel was: which is the greater effect, change in local grey matter volume, or change in benzodiazepine receptor density? In essence, the analysis was a Group $\times$ Modality interaction within SPM, the ‘Group’ referring to patients or normal control subjects, and ‘Modality’ being imaging modality (MRI or PET).

In order for this analysis to be possible there must be a consistent relationship, over subjects, between grey matter volume and FMZVD at each voxel. However, the measure of FMZVD per unit grey matter volume could be variable across brain regions. In order to compare the two sets of images the variance at each voxel should be similar for the two imaging modalities. We assumed a consistent correlation between grey matter volume and FMZVD at each voxel. Proportional scaling was used to achieve global normalization of voxel values between scans, to render the variance at each voxel the same in the two sets of data.

Two contrasts can be specified when testing for an interaction between the MRI and PET datasets: regions showing unexpectedly high FMZVD compared with grey matter volume, and regions showing unexpectedly low FMZVD compared with grey matter volume. These results can be compared with the comparison of individual FMZVD images with the normal control images to reveal those areas of brain in which apparently significant differences in FMZVD were due to abnormalities of local grey matter volume. Regions were defined with a threshold of $P < 0.01$. Regional abnormalities were retained only if they were significant after a correction for multiple comparisons (Friston et al., 1994), using $P < 0.05$ for the analysis of MRIGM and FMZVD images in isolation and $P < 0.5$ for the interactions. A low threshold was chosen for the interactions to ensure a low type II error in detecting mismatch between tracer uptake and grey-matter density. Since heterotopic nodules were excluded from the MRIGM images by virtue of the segmentation process, any result showing a significant interaction confined only to heterotopic nodules (present in the FMZVD images but not in MRIGM images) was excluded. The method is summarized in Fig. 1.

**Results**

Clinical details of the patients are summarized in Table 1.
Fig. 1 An outline summary of the method of comparison of PET and MRI, to allow detection of regions of abnormal FMZVD (flumazenil volume of distribution) per volume of grey matter. MRIGM = MRI-defined cortical grey matter.
**Table 1** Clinical characteristics, MRI, EEG and PET findings for 10 patients with malformations of cortical development

<table>
<thead>
<tr>
<th>Patient</th>
<th>MRI finding</th>
<th>Age</th>
<th>Epilepsy duration (years)</th>
<th>Sex</th>
<th>Seizures</th>
<th>Intercital EEG features</th>
<th>Drugs (daily dose in mg)</th>
<th>Time since last seizure</th>
</tr>
</thead>
<tbody>
<tr>
<td>M.C.</td>
<td>Bilateral cortical dysgenesis</td>
<td>18</td>
<td>17</td>
<td>F</td>
<td>L arm focal motor + SG</td>
<td>Bilateral independent foci (ictal)</td>
<td>LTG 400; CBZ 1000</td>
<td>2 days</td>
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<td></td>
<td>heterotopic nodules</td>
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<tr>
<td>A.F.</td>
<td>Subependymal heterotopia</td>
<td>47</td>
<td>21</td>
<td>F</td>
<td>Complex partial seizures + SG tonic</td>
<td>Bilateral temporal foci</td>
<td>PHT 375</td>
<td>2 months</td>
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<tr>
<td>R.H.</td>
<td>Bilateral cortical dysgenesis</td>
<td>34</td>
<td>33</td>
<td>F</td>
<td>L arm focal motor + SG</td>
<td>No definite abnormality</td>
<td>CBZ 800</td>
<td>2 days</td>
</tr>
<tr>
<td></td>
<td>and schizencephaly</td>
<td></td>
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<tr>
<td>T.J.</td>
<td>Bilateral band heterotopia</td>
<td>30</td>
<td>18</td>
<td>M</td>
<td>R arm focal motor + SG</td>
<td>L hemisphere slow</td>
<td>CBZ 600</td>
<td>4 days</td>
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<tr>
<td>S.N.</td>
<td>Single R posterior parietal</td>
<td>26</td>
<td>7</td>
<td>F</td>
<td>Simple partial seizures</td>
<td>No definite abnormality</td>
<td>CBZ 700</td>
<td>4 years</td>
</tr>
<tr>
<td></td>
<td>parietal heterotopic nodule</td>
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<tr>
<td>T.P.</td>
<td>Temporoparietal cortical dysgenesis</td>
<td>29</td>
<td>27</td>
<td>F</td>
<td>Complex partial seizures + SG</td>
<td>R posterior hemisphere focus</td>
<td>VPA 2500; CBZ 600;</td>
<td>8 days</td>
</tr>
<tr>
<td></td>
<td>and heterotopias</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td>LTG 100</td>
<td></td>
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<tr>
<td>M.S.</td>
<td>Bilateral band heterotopia</td>
<td>33</td>
<td>31</td>
<td>M</td>
<td>Complex partial seizures + SG</td>
<td>Irregular generalised spikes</td>
<td>ESM 1000; PHT 350;</td>
<td>36 h</td>
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<td></td>
<td>LTG 100</td>
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<tr>
<td>J.W.</td>
<td>L frontal and R temporal</td>
<td>37</td>
<td>13</td>
<td>F</td>
<td>SG tonic</td>
<td>Bilateral independent foci</td>
<td>GBP 1200; CBZ 1300</td>
<td>6 h</td>
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<tr>
<td></td>
<td>cortical dysgenesis</td>
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<tr>
<td>K.S.</td>
<td>Subependymal heterotopia</td>
<td>30</td>
<td>6</td>
<td>F</td>
<td>R arm focal sensory + SG</td>
<td>Normal</td>
<td>VPA 600; PHT 300</td>
<td>36 h</td>
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<tr>
<td>S.H.</td>
<td>Small nodule in post. L</td>
<td>29</td>
<td>14</td>
<td>F</td>
<td>L arm focal motor + SG</td>
<td>Generalised spike wave</td>
<td>CBZ 1000</td>
<td>3 days</td>
</tr>
<tr>
<td></td>
<td>parietal white matter</td>
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R = right; L = left; GVG = vigabatrin; PHT = phenytoin; CBZ = carbamazepine; PMD = primidone; ESM = ethosuximide; LTG = lamotrigine; VPA = sodium valproate; GBP = gabapentin; SG = secondarily generalized.

**SPM analysis of MRI structural data and comparison with region-based morphometrics**

Comparison of each normal control MRIGM image with those of the remaining 15 normal control subjects revealed only one significant region in one individual at the statistical threshold of $P < 0.001$, corrected $P < 0.05$. Since 32 tests were made (examination for regions of increased grey matter volume and examination for regions of decreased volume in each of 16 subjects) at least one significant region was expected by chance.

Five of the 10 patients had abnormal block volumes according to the region-based morphometrics; the approximate positions of these abnormal blocks are marked in Fig. 1. Since the block-volume measurements were performed on data which were not spatially normalized, the positions of the blocks were approximately indicated by dividing the spatially normalized brain into 10 equal coronal partitions. Several measures were made in each block (grey matter volume, white matter volume and both within-block ratios and ratios between the block volumes and those of the opposite hemisphere homotopic block). We have not separated the various categories of block abnormality since the different measures are not necessarily independent.

Using SPM and a threshold of $P < 0.05$ (corrected) with ANCOVA, the same five patients were found to have abnormalities of MRIGM. The remaining five patients were found to be normal by both techniques. The SPM results are displayed next to the block-volume results for comparison in Fig. 1. Although some abnormalities are anatomically concordant, particularly in M.C., R.H. and J.W., others are not. However, using these two methods, the concordance in terms of detection of an abnormal brain is striking. SPM permitted the identification of regions of increased or decreased grey matter volume with respect to control subjects in greater anatomical detail compared with region-based morphometrics.

**Comparison of structural and functional images**

Figure 2 shows the SPM results of analysis of the MRIGM data displayed alongside the SPM analysis of the FMZVVD data; the results of the analysis of the interaction between these two imaging modalities is also shown. For all of these analyses proportional scaling was used and regions were defined using a threshold of $P < 0.01$, significant regions are reported at $P < 0.05$ for the within-modality comparisons and at $P < 0.5$ for the interaction. In two cases (S.H. and K.S.) no abnormalities were found with either modality and no significant interaction was revealed. In two further cases (T.J. and T.P.) significant abnormalities in one imaging modality were associated with similar abnormalities in the other modality such that no regions of abnormal FMZVVD per grey matter volume were identified; T.J. had a region of increased FMZVVD in the right central region, accounted for by increased grey matter volume in that area; T.P. had a region of decreased FMZVVD in the right posterior temporal/occipital area, accounted for by a decrease in grey matter volume in that area. In both of these cases there were other...
Fig. 2 Results of the analyses of cortical grey matter volumes. For each patient the approximate position of the abnormal blocks using the volume-of-interest block volume measurement technique are shown (left column), as are the significant regions of increased (middle column) and decreased grey matter volume (right column) using SPM. The results are displayed as maximum intensity projections (as though viewing the regions within a ‘glass brain’) in the sagittal, coronal and transverse planes; left of the brain is on the left of the image. Regions were deemed significant at $P < 0.001$ with a correction for multiple non-independent comparisons at $P < 0.05$. 
<table>
<thead>
<tr>
<th>Subject</th>
<th>Increased MRGGM $P&lt;0.01$</th>
<th>Decreased MRGGM $P&lt;0.01$</th>
<th>Increased FMZVD $P&lt;0.01$</th>
<th>Decreased FMZVD $P&lt;0.01$</th>
<th>Relatively high FMZVD $P&lt;0.01$</th>
<th>Relatively low FMZVD $P&lt;0.01$</th>
<th>EEG focus</th>
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<tbody>
<tr>
<td>M.C.</td>
<td><img src="image1.png" alt="Images" /></td>
<td><img src="image2.png" alt="Images" /></td>
<td><img src="image3.png" alt="Images" /></td>
<td><img src="image4.png" alt="Images" /></td>
<td><img src="image5.png" alt="Images" /></td>
<td><img src="image6.png" alt="Images" /></td>
<td>Bilateral widespread epileptiform abnormality</td>
</tr>
<tr>
<td>A.F.</td>
<td><img src="image7.png" alt="Images" /></td>
<td>No significant voxels</td>
<td><img src="image8.png" alt="Images" /></td>
<td><img src="image9.png" alt="Images" /></td>
<td><img src="image10.png" alt="Images" /></td>
<td><img src="image11.png" alt="Images" /></td>
<td>Bilateral anterior temporal spikes</td>
</tr>
<tr>
<td>R.H.</td>
<td><img src="image12.png" alt="Images" /></td>
<td><img src="image13.png" alt="Images" /></td>
<td><img src="image14.png" alt="Images" /></td>
<td><img src="image15.png" alt="Images" /></td>
<td><img src="image16.png" alt="Images" /></td>
<td><img src="image17.png" alt="Images" /></td>
<td>No definite abnormality</td>
</tr>
<tr>
<td>T.J.</td>
<td><img src="image18.png" alt="Images" /></td>
<td>No significant voxels</td>
<td><img src="image19.png" alt="Images" /></td>
<td><img src="image20.png" alt="Images" /></td>
<td><img src="image21.png" alt="Images" /></td>
<td><img src="image22.png" alt="Images" /></td>
<td>Left hemisphere slow</td>
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<tr>
<td>S.N.</td>
<td><img src="image23.png" alt="Images" /></td>
<td>No significant voxels</td>
<td><img src="image24.png" alt="Images" /></td>
<td><img src="image25.png" alt="Images" /></td>
<td><img src="image26.png" alt="Images" /></td>
<td><img src="image27.png" alt="Images" /></td>
<td>No definite abnormality</td>
</tr>
<tr>
<td>T.P.</td>
<td><img src="image28.png" alt="Images" /></td>
<td>No significant voxels</td>
<td><img src="image29.png" alt="Images" /></td>
<td><img src="image30.png" alt="Images" /></td>
<td><img src="image31.png" alt="Images" /></td>
<td><img src="image32.png" alt="Images" /></td>
<td>Right posterior spikes</td>
</tr>
<tr>
<td>M.S.</td>
<td><img src="image33.png" alt="Images" /></td>
<td><img src="image34.png" alt="Images" /></td>
<td><img src="image35.png" alt="Images" /></td>
<td><img src="image36.png" alt="Images" /></td>
<td><img src="image37.png" alt="Images" /></td>
<td><img src="image38.png" alt="Images" /></td>
<td>Irregular generalized spikes</td>
</tr>
<tr>
<td>J.W.</td>
<td><img src="image39.png" alt="Images" /></td>
<td><img src="image40.png" alt="Images" /></td>
<td><img src="image41.png" alt="Images" /></td>
<td><img src="image42.png" alt="Images" /></td>
<td><img src="image43.png" alt="Images" /></td>
<td><img src="image44.png" alt="Images" /></td>
<td>Bilateral fronto-temporal spikes</td>
</tr>
</tbody>
</table>
areas of abnormal grey matter volume which were associated with similar changes in FMZVD in those areas which, while non-significant when examined alone, were sufficient to exclude any significant interaction.

The remaining six cases all showed significant interaction effects, with regions of significantly abnormal FMZVD per grey matter volume. Five of the seven subjects found to have abnormalities of FMZVD examined in isolation (M.C., A.F., R.H., M.S. and J.W.) were shown to have a significant interaction between MRIGM and FMZVD, demonstrating that, in these cases, the abnormal FMZVD is not merely a consequence of abnormal anatomy, but reflects abnormal benzodiazepine receptor density in these grey matter regions. However, this was not the case for all areas in these subjects. For example, the cingulate gyrus in J.W. and left parietal cortex in R.H., which were found to show abnormal FMZVD examined in isolation, were found to have a proportionate change in grey matter volume in these areas. Specifically, in the case of J.W., increased cingulate FMZVD was accounted for by an unsuspected increase in grey matter volume in this area. In the case of R.H., decreased left parietal cortex FMZVD was accounted for by an unsuspected decrease in grey matter volume in this area.

In one subject, S.N., interaction analysis showed an abnormality of \([^{11}\text{C}]\text{flumazenil}\) that was not evident on analysis of the FMZVD data in isolation: the left prefrontal area showed an increase in grey matter volume, but no corresponding increase in FMZVD. Even in those subjects in whom both abnormality of MRIGM and abnormality of FMZVD was seen when analysed in isolation, the interaction analysis provided ‘added value’. For example, in R.H., a schizencephalic cleft is seen in the left central area, reflected by increased MRIGM and increased FMZVD in this area. The interaction analysis shows, unexpectedly, that the posterior lip of the cleft has reduced \([^{11}\text{C}]\text{flumazenil}\) binding per grey matter volume, and the anterior lip has increased \([^{11}\text{C}]\text{flumazenil}\) binding. Further, in subject M.S. there are no regions of decreased FMZVD, but there are several regions of decreased grey matter volume; the interaction analysis reveals that some of these areas of decreased grey matter volume have relatively high \([^{11}\text{C}]\text{flumazenil}\) binding, which was not apparent in the analysis of FMZVD images alone.

Patients S.N., T.P. and K.S. had significantly high FMZVD compared with MRIGM in the region of heterotopic nodules only. These results were artefacts consequent upon the exclusion of heterotopic nodules from the MRIGM images, and are not shown.

**Discussion**

**Voxel-based analysis of MRI using SPM**

We have shown that five out of 10 patients with malformations of cortical development and localization-related epilepsy had abnormalities of regional cerebral volumes as measured in coronal blocks. We have shown that these same five patients had abnormalities of regional grey matter volume detected using SPM. The remaining five patients were found to be normal by both techniques. The specificity of SPM in this context was demonstrated by the finding that only one of the sixteen normal control subjects was found to have a single region of abnormal MRIGM in comparison with the remaining 15 control subjects. This chance finding was expected at a corrected threshold of $P < 0.05$. SPM permits an anatomical localization of regional abnormalities of cortical grey matter volume at a resolution greatly superior to the block volume method. In addition, SPM is entirely objective and is not constrained by predetermined volumes-of-interest. The finding of an abnormality of a block volume does not allow the location of this abnormality to be pinpointed. The analysis of structural MRI data using SPM may be a useful technique in the investigation of patients with epilepsy as well as other neurological and psychiatric disease, particularly to delineate the extent of a structural abnormality, or when no abnormality is evident by visual inspection.

**Voxel-based comparison of MRI and PET in malformations of cortical development using SPM**

We have also shown that six out of 10 patients showed regions of cerebral cortex with disproportionate flumazenil binding compared with local grey matter volume, by formally testing for an interaction effect. Five of these patients had regional abnormalities of FMZVD on examination of the FMZVD images alone. These included both areas of increased FMZVD as well as areas of decreased FMZVD. In addition, we have shown that one patient with abnormalities of MRIGM, but no abnormality of FMZVD examined alone, showed abnormal FMZVD when compared with MRIGM. Finally, no patient with both MRIGM and FMZVD images found to be normal when examined in isolation was found.

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**Fig. 3** Results of the comparison of MRIGM (MRI-defined cortical grey matter) and FMZVD (flumazenil volume of distribution) images using SPM to detect a significant interaction effect. For each subject the significant regions of increased and decreased grey matter volume examined in isolation, the significant regions of increased and decreased flumazenil binding examined in isolation, and the regions of disproportional increase and decrease flumazenil binding (compared with grey matter volume) are shown. The results are displayed as maximum intensity projections (as though viewing the regions within a ‘glass brain’) in the sagittal, coronal and transverse planes; left of the brain is on the left of the image. Regions were deemed significant at $P < 0.01$ (a lower threshold than in Fig. 1) with a correction for multiple non-independent comparisons at $P < 0.05$ for the within-modality analyses and $P < 0.5$ for the interaction.
to have any disproportionate flumazenil binding. The identification of regions of increased flumazenil binding in patients with malformations of cortical development, when abnormalities of cortical volume have been taken into account, confirms our previous finding of increased flumazenil binding when FMZVD images were examined in isolation (Richardson et al., 1996b). This might be explained by either an increased density of neurons bearing GABA<sub>A</sub> receptors, an increased density of these receptors per neuron, or an increased affinity of these receptors. Other potential explanations for the finding of increased flumazenil binding in patients with epilepsy included abnormalities of grey matter structure, or possibly an artefactual consequence of global normalization of FMZVD data. Since both anatomical abnormality and global normalization would have the same effect in MRIGM images as in FMZVD, direct comparison of these two modalities allows us to exclude these effects. In the majority of cases, we found that regions of relatively low or high flumazenil binding remained when these factors were taken into account. Therefore, the finding of regions of increased flumazenil binding, as well as decreased flumazenil binding per unit volume of grey matter, is a real phenomenon. In some cases, however, abnormalities of FMZVD images examined in isolation were shown to be accounted for simply by subtle and previously unnoticed abnormalities of grey matter volume. For example, R.H. had a region of low FMZVD in the left parietal lobe; in the same area there was a region of reduced MRIGM. As a result, no disproportionate flumazenil binding was seen in that area. Similarly, increased FMZVD was observed in the cingulate of J.W.; increased MRIGM was also seen in this area, such that no disproportionate flumazenil binding was detected.

No individual with both normal FMZVD and normal MRIGM examined independently had any disproportionate flumazenil binding when the two modalities were compared. The normal control group for MRIGM was composed of different individuals from the normal control group for FMZVD. We have established in the current study that the detection of abnormality of MRIGM in normals is exceptional. We have already shown that abnormalities of FMZVD in normal control subjects are also very rare (Richardson et al., 1996b). It is an assumption of this study that both anatomy and function would be normal in all of the normal control subjects, although only one modality of imaging has been performed in each normal subject. It is reassuring that no patient with normal MRI and PET imaging, analysed independently, showed any significant abnormalities when the structural and functional data were compared directly.

**Voxel-based comparison of MRI and PET: general methodological considerations**

Several important general issues are addressed by these findings. It might be thought that the processes of volumetric normalization and smoothing of images might artefactually create apparent anatomical abnormalities in normal brain images, or remove real abnormalities from abnormal images. We have demonstrated that neither of these phenomena occur. In total, 26 individuals were studied; five were found to be abnormal by a rigorous and meticulous regional volume measurement technique; the same five were designated abnormal by SPM.

The technique allowed for inferences about abnormalities in FMZVD per grey matter volume in a way that avoids using the ratio itself. This was achieved by testing for interactions. This is important because alternative approaches based explicitly on the ratio would not lend themselves to parametric statistical analysis (because quotients of this sort are not normally distributed). In order to combine the two modalities of data implicitly in a statistical model we have to ensure they have similar distributions under the null hypothesis. In this case we have used proportional scaling by the whole brain mean to achieve this.

A crucial feature of our method is the use of proportional scaling for global normalization of voxel values. Assuming a constant relationship between MRIGM and FMZVD in each brain region, global normalization by proportional scaling also leads to a proportional scaling of the error variance such that homoscedasticity is assured. The interaction effect can only be examined under this condition. The assumption of a constant relationship between MRIGM and FMZVD in each brain region is entirely reasonable given that (under normal circumstances) tracer uptake will be proportional to receptor density, which will in turn be proportional to local grey matter volume. We have shown that differences between normal control subjects in each modality are hard to detect, therefore there is a predictable distribution of values at each voxel in the two modalities. It is also important to ensure the spatial autocovariance structure of the two data sets is the same. This can be achieved by differential smoothing to the same smoothness; the method of estimating smoothness has been described elsewhere (Poline et al., 1995). It is reassuring to note that in an empirical study of FMZVD images alone using a voxel-based comparison, in this case of two normal groups, the error terms were found to be normally distributed (Frey et al., 1996).

We have also demonstrated the feasibility of using different templates for different imaging modalities while maintaining exact registration of the two normalized images for the same individual. This is partly demonstrated by the point above; misregistration would result in artefactual differences between the two imaging modalities in individuals with normal imaging in each modality considered independently. It is also demonstrated by the observation that, in a number of cases, abnormalities in one modality are precisely counterbalanced by abnormality of the other modality in the same anatomical region. Abnormalities of FMZVD and MRIGM would only be seen to 'cancel out' if the coregistration was adequate.

It should be noted that the analysis of flumazenil binding...
in relation to grey-matter density is based upon a statistical interaction between patient (versus normal group) and modality of imaging. In the design described here the data for the two modalities were obtained from two separate normal control groups. A more powerful design would be to use the same subjects for both the PET and MRI scans. The subject-specific effects could then be modelled for both the normal control subjects and the patients, therein increasing the power of the analysis by removing a further component of error variance. Although our current analysis is valid, it may be conservative in relation to this alternative.

A number of criticisms could be made of this method. First, MRIGM images were assigned a single constant voxel value, given to all regions of cortex before smoothing; benzodiazepine receptor density is known not to be constant across all grey matter regions in human brain by autoradiographic mapping (Zezula et al., 1988). Although gross differences in receptor density over the brain may violate the assumption of homoscedasticity above, they are not, in themselves a problem for the technique. This is because a low (or high) receptor density will result in a difference between FMZVD and MRIGM means only i.e. a main effect of modality. However, FMZVD per unit grey matter differences represent interactions and are not affected by this main effect. This argument assumes, of course, that the error variances are the same for the FMZVD and MRIGM data after normalization. If this is not the case, more sophisticated normalization transformations may be required (e.g. by weighting every segmented image by the mean FMZVD image over all subjects). Secondly, no account has been taken of the contribution to apparent FMZVD values in cortex by 'spill-in' from activity in surrounding white matter. However, it has been shown that benzodiazepine receptor binding in white matter is negligible compared with that in the cortex (Zezula et al., 1988). For a ligand with significant white-matter binding, the assumption of a non-grey value of zero in the MRIGM images would have to be re-evaluated. A more sophisticated approach would be to combine grey, white and CSF segmented image partitions linearly in a way that best predicted the mean FMZVD image in a least-squares sense. Finally, the method outlined here has still required interactive segmentation of the MRI data by a skilled investigator. This is labour-intensive and somewhat subjective, although our data has been shown previously to have been segmented with good inter- and intra-operator reliability (Sisodiya et al., 1995). Automated MRI segmentation techniques now available as part of SPM96 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) should enable this step of the analysis to be automated and rendered entirely objective.

Although voxel-based comparison of MRI and PET using SPM has many advantages, particularly the automated and objective analysis of all brain regions, this method may have some disadvantages. The spatial transformation of images and normalization to global mean enable relative regional changes to be detected, but at the expense of the detection of global change and the measurement of absolute quantitative values. A regions-of-interest based approach, though subjective in terms of region placement, and limited to the regions selected, allows absolute quantification. Using high-resolution MRI and the segmentation of the imaging data into three compartments (grey matter, white matter and cerebrospinal fluid), a model has been described to determine functional changes per unit volume of grey matter tissue in regions-of-interest (Muller Gartner et al., 1992; Labbe et al., 1996). Recently the three-compartment model has been extended to include a fourth, grey matter region-of-interest compartment, which could be delineated on MRI and had a local tissue concentration different from the rest of the grey matter (Frost et al., 1995). Despite the improvement in accuracy of parameter quantification provided by these methods, the three-compartment model is inadequate because homogeneity of grey matter is assumed and the four compartment model requires the three-compartment solution to be calculated first, leading to accumulated error. Further, only limited numbers (one in the four compartment model) of volumes-of-interest can be examined. The approach described here can be seen as addressing the same issues in a simpler and statistically rigorous manner throughout the entire brain volume. Rather than being seen as 'rival' techniques, region-of-interest- and voxel-based methods may be better seen as complementary; SPM could be used to compare structural and functional images in order to detect regions of significant abnormality and to determine whether any functional abnormality could be explained by structural abnormality in that region. These significant regions could then be analysed with a region-of-interest technique employing partial volume effect correction (Muller Gartner et al., 1992; Rousset et al., 1993; Frost et al., 1995; Rousset et al., 1995; Labbe et al., 1996) to obtain absolute quantitative values.

In conclusion, we have demonstrated the validity of a voxel-based technique for the comparison of individual structural images with a normal group of such images, which allows subtle structural abnormalities to be located. We have previously described the application of voxel-based methods to the comparison of individual PET ligand images with a group, which allows functional abnormalities to be located. Building on these two approaches, we have described a novel technique for the direct comparison of structural and functional images using SPM, which allows issues concerning partial volume effects and mixed tissue sampling in PET ligand images to be resolved. This is pertinent to any ligand PET investigation in which either atrophy of a structure or increase in volume, as in malformations of cortical development, may be encountered. Using this method we have demonstrated that although patients with malformations of cortical development show changes in cortical volume and changes in FMZVD, these changes are often disproportionate; six out of 10 of our patients showed abnormalities of FMZVD which were not simply accounted for by changes in cortical volume. The abnormality of FMZVD per grey matter volume detected by this method
demonstrates the existence of a functional abnormality in patients with malformations of cortical development in addition to structural abnormality. This functional abnormality could be due to abnormal neuronal density or abnormal numbers or affinity of benzodiazepine receptors. The analysis of ligand PET data should always include a comparison with structural data, in the form of high-resolution MRI, which is greatly facilitated by the approach described here.

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References


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