Abnormal cerebral structure in juvenile myoclonic epilepsy demonstrated with voxel-based analysis of MRI

F. G. Woermann, S. L. Free, M. J. Koepp, S. M. Sisodiya and J. S. Duncan

The MRI Unit, National Society of Epilepsy and Epilepsy Research Group, University Department of Clinical Neurology, Institute of Neurology, London, UK

Correspondence to: Professor J. S. Duncan, National Society for Epilepsy, National Hospital for Neurology and Neurosurgery, Chalfont St Peter, Gerrards Cross, Bucks SL9 0RJ, UK

Summary
MRI scans of patients with idiopathic generalized epilepsy (IGE) are normal on visual assessment. Using an interactive anatomical segmentation technique and volume-of-interest measurements of MRI, we showed recently that patients with IGE had significantly larger cortical grey matter than control subjects. Further, 40% of individual patients with juvenile myoclonic epilepsy (JME), a syndrome of IGE in adolescence, had significant abnormalities of cerebral structure. In this study, we applied the automated and objective technique of statistical parametric mapping (SPM) to the analysis of structural MRI from 20 patients with JME and 30 control subjects. The cortical grey matter of each individual JME patient and the group of JME patients was contrasted with that of the group of 30 normal subjects. The voxel-based SPM comparison between the group of JME patients and the control subjects showed an increase in cortical grey matter in the mesial frontal lobes of the patients. Analysis of individual patients revealed significant abnormalities of cortical grey matter in five out of 20 JME patients, four of whom had been shown to have widespread abnormalities using the previous volume of interest technique. These findings indicate a structural cerebral abnormality in JME, with involvement of mesiofrontal cortical structures.

Keywords: idiopathic generalized epilepsy, MRI, voxel-based morphometry

Abbreviations: IGE = idiopathic generalized epilepsy; JME = juvenile myoclonic epilepsy; SPM = statistical parametric mapping

Introduction
Juvenile myoclonic epilepsy (JME) is a syndrome of idiopathic generalized epilepsy (IGE) with an age-related onset of seizures; it is characterized by myoclonic jerks, tonic–clonic seizures and less frequently by typical absences. Prevalence is 5–10% among adult and adolescent patients with epilepsies, and both sexes are equally affected (Janz and Durner, 1998). As with other IGE syndromes, JME is defined by electrophysiological features indicating involvement of both cerebral hemispheres from the beginning of seizures. There are ‘no neuroradiological signs’ in patients with JME (Commission on Classification, 1985) and visual inspection of high-resolution MRI is normal in patients with IGE. However, using semi-automated MR segmentation and quantitation, we identified widespread cerebral structural changes with larger than normal cortical grey matter volumes in patients with various IGE syndromes, supporting the existence of structural changes in these patients (Woermann et al., 1998). The spatial resolution of this study was constrained by the use of volumes of interest that comprised one-tenth of the anteroposterior extent of each cerebral hemisphere.

The autopsy studies of Janz and Meencke (Janz and Neimanis, 1961; Meencke and Janz, 1984; Meencke, 1985) have shown cortical and subcortical dystopic neurons and other microscopic structural abnormalities (‘microdysgenesis’) in a large percentage of a small number of patients with IGE, some of whom had JME. These findings are controversial (Lyon and Gastaut, 1985). Quantitative MRI may be used as a surrogate for histology to examine the whole brain in detail in vivo (Sisodiya et al., 1995; Sisodiya and Free, 1997; Richardson et al., 1997). The aim of the current study was to identify subtle, structural abnormalities of the neocortex in patients with JME, using an anatomically specific and objective voxel-by-voxel analysis of segmented cortical grey matter.
Methods

Subjects
We studied 20 patients with JME (12 women, eight men; median age 25 years, range 15–37 years) who were recruited from the epilepsy clinics of the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK. Median age of onset of JME was 14 years (range 7–21 years). All patients suffered from myoclonic jerks and generalized clonic–tonic seizures. Nine patients also had typical absences. At the time of the study 12 patients were seizure-free whilst being treated with antiepileptic drugs. EEG recordings showed generalized epileptiform activity in 18 patients (90%), comprising generalized discharges of spikes and polyspike and slow waves; eight patients also demonstrated 3- to 4-Hz generalized spike wave complexes. Epileptiform activity was induced by photic stimulation in six patients (30%). The control group comprised 30 subjects who had no history of any neurological or psychiatric disorders (16 women, 14 men; median age 27 years, range 14–36 years). Written informed consent was obtained from all subjects. Approval for the study was obtained from the ethical committee of the National Hospital for Neurology and Neurosurgery.

MRI scanning protocol
MRI was performed using a 1.5 T GE Signa scanner (Milwaukee, Wis., USA). An inversion recovery-prepared 3D Spoiled Gradient Echo (IRP-SPGR) sequence (TR = 17.4 ms, TE = 4.2 ms, TI = 450 ms, flip angle = 20°, matrix size 256 × 192, field of view 24 × 18 cm) with 124 contiguous coronal slices and a slice thickness of 1.5 mm was used for volumetric studies. All MRIs were reviewed by experienced neuroradiologists and reported as normal for using two contrasts (more or less grey matter in patients compared with controls). This analysis detected whether each voxel in the control group, and in this way revealed excess or reduction of grey matter in patients. The resulting set of voxel values for each contrast constituted a statistical parametric map of the t statistic (SPM{t}). The SPM{t} values were transformed to the normal distribution (SPM{Z}) and thresholded at $P < 0.001$. To correct for multiple comparisons, the resulting foci were characterized in terms of the differences in intensity and spatial extent (Friston et al., 1994). This correction described the probability that a region of the observed intensity difference and size could have occurred by chance over the entire volume analysed (i.e. a corrected $P$ value). The corrected $P$ value chosen was $P < 0.05$.

Segmentation of MRI and MRI volumetry
For segmentation of cortical grey matter, MRIs were transferred to an independent image analysis workstation (Allegro; ISG Technologies, Toronto, Canada), allowing the semiautomated selection of defined regions of interest from the contiguous coronal MRIs. This segmentation technique employed interactive thresholding of signal intensity between the grey matter and CSF and between the grey matter and white matter, and used an automated seed-growing algorithm as previously published (Sisodiya et al., 1995; Woermann et al., 1998). Total cerebral volume and cortical grey matter volumes from each hemisphere were constructed and measured. The cortical grey matter volumes were used for subsequent voxel-by-voxel analysis (Fig. 1A and B). Data from the previous study describing cerebral volume of interest in the same JME patients and controls (Woermann et al., 1998) were used for comparison with results from the voxel-by-voxel comparison of this study.

Data analysis
Data were analysed on a Sun SPARC 20 workstation (Sun Microsystems, Mountain View, Calif., USA) using Analyze version 7.5 [Mayo Foundation (Robb et al., 1989)], MATLAB 4.2a (MathWorks, Natick, Mass., USA) and SPM (Statistical Parametric Mapping) 96 (Wellcome Department of Cognitive Neurology, Institute of Neurology, London, UK) (Friston et al., 1995a, b).

Analysis of structural MRI data in SPM
A binarized image of the entire MRI-defined and semiautomatically segmented cortical grey matter from both cerebral hemispheres was used for the analysis with SPM. SPM characterizes significant regional differences in images whilst taking account of global differences. Firstly, the individual T1-weighted MRIs (i.e. the images before segmentation) were transformed into standard 3D space without requiring user-defined landmarks, using the T1-weighted MRI template of SPM 96. The default spatial normalization of SPM 96 used linear and non-linear transformations. These normalization parameters were then applied to the binarized grey matter image of the same person; these normalized grey matter images were then smoothed with a 14 mm full width at half maximum isotropic Gaussian kernel (Fig. 1C and D). Spatial smoothing was used to allow for interindivdual gyral variation, so that individual brains of the control group were not identified as abnormal. The regional voxel intensity of the smoothed images reflected the probability of voxels being grey matter. To normalize for differences in voxel intensity across scans, the global mean voxel value was included as a confounding covariate in an ANCOVA (analysis of covariance) (Friston et al., 1990). To test hypotheses about regionally specific effects in the imaging data, the estimates were compared using two contrasts (more or less grey matter in patients compared with controls). This analysis detected whether each voxel had a greater or lesser probability of being grey matter in a patient or the patient group than the same localized voxel in the control group, and in this way revealed excess or reduction of grey matter in patients. The resulting set of voxel values for each contrast constituted a statistical parametric map of the t statistic (SPM{t}). The SPM{t} values were transformed to the normal distribution (SPM{Z}) and thresholded at $P < 0.001$. To correct for multiple comparisons, the resulting foci were characterized in terms of the differences in intensity and spatial extent (Friston et al., 1994). This correction described the probability that a region of the observed intensity difference and size could have occurred by chance over the entire volume analysed (i.e. a corrected $P$ value). The corrected $P$ value chosen was $P < 0.05$.

Results

Volumetric measurements
In control subjects, the mean total cerebral volume was 1017 cm$^3$ (SD = 128 cm$^3$). There was a gender-related difference
Abnormal cerebral structure in JME

Fig. 1 A summary of the method of comparison of neocortical grey matter. Grey matter on T1-weighted IR-SPGR images (A) was segmented semiautomatically, resulting in a grey matter image (B). These images were normalized (C; note the slight changes in orientation and relative size) and smoothed (D), and then compared with images for 30 control subjects. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the \( t \) statistic (SPM{\( t \)}). The SPM{\( t \)} values were transformed to normal distribution (SPM{\( Z \)}) (E; shown here for an increase in grey matter). After correction for multiple comparisons the resulting sagittal, coronal and axial 'glass brain' views show voxels with a significant increase in grey matter in this single JME patient compared with 30 control subjects (thresholded at \( P < 0.05 \)) (F). This patient had no significant clusters of decreased grey matter.

in overall cerebral volume. The mean cerebral volume in females (\( n = 16 \)) was 961 cm\(^3\) (SD 96 cm\(^3\)) while that in males (\( n = 14 \)) was 1081 cm\(^3\) (SD = 134 cm\(^3\)) (Mann–Whitney \( U \) test, \( P < 0.01 \)).

In 20 JME patients, the mean total cerebral volume was 960 cm\(^3\) (SD = 90 cm\(^3\)). The mean cerebral volume in female patients (\( n = 12 \)) was 918 cm\(^3\) (SD = 72 cm\(^3\)), which was significantly less than that in males (1022 cm\(^3\); \( n = 8 \)), (SD = 80 cm\(^3\)) (Mann–Whitney \( U \) test, \( P < 0.01 \)). There was no difference in mean total cerebral volume between all patients and controls (Mann–Whitney \( U \) test, \( P > 0.1 \)), between female controls and female JME patients (Mann–Whitney \( U \) test, \( P > 0.1 \)) or between male controls and male JME patients (Mann–Whitney \( U \) test, \( P > 0.2 \)).

Voxel-by-voxel analysis: defining a normal range

Single-case voxel-by-voxel SPM analysis of the cortical grey matter distribution of each normal subject with those of the remaining 29 normal control subjects revealed one significant abnormal region in each of two individuals at the statistical threshold of \( P < 0.001 \), corrected \( P < 0.05 \): one control subject had a bilateral temporopolar, frontobasal decrease, the other a bitemporolateral increase of grey matter. Since 60 tests were made (examination for regions of increased and decreased grey matter volume), three abnormal areas would be expected by chance at a significance level of \( P < 0.05 \), and therefore our control data with two abnormal areas identified by SPM provided a valid control group for comparison with the patient data. There was no difference in the distribution of cortical grey matter of 16 female compared with 14 male control subjects.

Single-case analysis of JME patients

Using the same corrected threshold of \( P < 0.05 \), five out of 20 JME patients had significant abnormalities when compared with the group of 30 controls. Two patients had bilateral areas of increased grey matter volume in temporoposterior
Fig. 2 Results of the analysis of cortical grey matter distribution, comparing grey matter of 20 JME patients with that of 30 control subjects. Results show significant regions of increased grey matter, which are displayed as maximum-intensity projections (as though viewing the regions within a glass brain) before (A) and after (B) correction for multiple comparisons (corrected \( P < 0.05 \)).

and mesioparietal regions (Fig. 1E and F) respectively, while three had areas of decreased grey matter volume, two patients showing this decrease in a frontopolar area and one frontomesially.

**Group analysis**

Comparing cortical grey matter volumes of the 20 JME patients with those of the 30 control subjects at a corrected threshold of \( P < 0.05 \), an area of increased grey matter was found in the patient group, with a maximum in the left mesiofrontal cortex (Talairach coordinates: \( x = -8 \text{ mm}, y = -6 \text{ mm}, z = 52 \text{ mm} \); superior frontal gyrus) (Fig. 2B). Before correction for multiple comparisons, these abnormalities were evident bilaterally in a pericallosal distribution (Fig. 2A). No significant decreases in grey matter were found in the JME group after correction for multiple comparisons.

Review of the results of individual patients in the light of this group finding showed that there were mesiofrontal increases in grey matter in 11 out of 20 JME patients, without corrections for multiple comparisons (as in Fig. 1E).

**Discussion**

Using the automated, objective technique of SPM, we found evidence for frontal grey matter abnormalities in patients with JME. Group voxel-by-voxel comparison of JME patients with control subjects using SPM revealed a mesiofrontal increase of grey matter in patients with JME. This builds on the findings of a recent volume of interest-based study, which demonstrated subtle widespread cerebral structural changes in patients with JME (Woermann et al., 1998) and adds localizing information and anatomical specificity to the group-to-group comparison. Comparing individual patients with a normal range, both the volume of interest and the voxel-based methods described structural abnormalities in a proportion of individual JME patients.

**Methodological considerations**

In patients with partial seizures and focal cortical dysgenesis that was detected visually on routine MRI, a previous study using both the volume of interest and the SPM method found a high degree of concordance in terms of detecting an abnormal grey matter structure, thus validating the voxel-by-voxel SPM approach to structural quantitative MRI (Richardson et al., 1997). In the present study, we applied the SPM analysis technique to the grey matter volumes of patients with JME and no visually detectable abnormality on MRI (‘MRI-negative’). Compared with studies of structural MRI using a voxel-based method in MRI-negative patients with schizophrenia or depression (Wright et al., 1995; Shah et al., 1998), we refined the approach by first establishing a normal range in 30 control subjects. We used the same grey matter volumes, segmented semiautomatically in our previous volume of interest-based study of the same patient and control groups, for the voxel-based comparison. We found that five out of 20 individual patients with JME had regional abnormalities of grey matter distribution, using SPM with a rigorous statistical threshold. The locations of these changes were different in different patients and from the mesiofrontal grey matter increase found in the group comparison (Table 1). This explains why changes seen in individual patients, such as decreases in frontal grey matter in three patients,
Table 1 Localization of grey matter abnormalities detected in five individual JME patients using SPM, compared with previous volumes of interest-based analysis (Woermann et al., 1998)

<table>
<thead>
<tr>
<th>Patient</th>
<th>SPM results</th>
<th>Results in coronally orientated volume of interest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bilateral temporoposterior increase</td>
<td>Bilateral parietotemporal increase</td>
</tr>
<tr>
<td>2</td>
<td>Left mesioparietal increase</td>
<td>Bilateral parietotemporal and frontal increase</td>
</tr>
<tr>
<td>3</td>
<td>Frontomesial decrease</td>
<td>No abnormalities</td>
</tr>
<tr>
<td>4</td>
<td>Bilateral frontopolar decrease</td>
<td>Bilateral frontal decrease</td>
</tr>
<tr>
<td>5</td>
<td>Bilateral frontopolar decrease</td>
<td>Bilateral frontal and frontotemporal decrease</td>
</tr>
</tbody>
</table>

were not seen in the group comparison after correction for multiple comparisons, which was not strongly affected by individual outliers. However, in 11 out of 20 individual JME patients analysed without correction for multiple comparisons, there were mesiofrontal increases (as in Fig. 1E). Although analyses have to be corrected for multiple comparisons to be valid, consideration of uncorrected results illustrates how a number of individual findings of low statistical significance can result in a significant group finding.

Four of these five JME patients also had regional abnormalities identified with the volume of interest method published previously (Woermann et al., 1998). The agreement in localization of abnormalities in individual patients was good in three of the four patients, with abnormalities identified using both the volume of interest and voxel-based methods, the latter being of superior spatial resolution and localizing potential (Table 1). The volume of interest method used in the previous study identified more individual JME patients’ brains as being structurally abnormal than did the SPM method. The differences in the results of the two methods are most likely due to the smoothing and thresholding used in the voxel-based method, with the consequence that some abnormalities in individual patients are likely to be below the threshold of detection. The volume of interest-based method also considered several derived variables (ratios of grey and white matter or ratios of left : right tissue volume). No histological correlation is available in this patient group.

The specificity of SPM in this context was demonstrated by the finding that only two of the 30 normal subjects had a single region of abnormal grey matter in comparison with the remaining 29 normal subjects; the locations of these two regions differed from one another and did not affect the mesial pericallosal area. This chance finding was expected at a corrected threshold of \( P < 0.05 \). The effectiveness of spatial normalization and smoothing to enable intersubject averaging and comparisons of homologous voxels was demonstrated by the SPM comparison of gender subgroups among our normal subjects, which showed no differences in the neocortical grey matter between the genders after image normalization and smoothing at the chosen significance level, although mean intracerebral volumes differed significantly between male and female controls. This finding is in keeping with earlier studies showing that possible gender differences were eliminated from MRI morphometric analysis by normalization procedures such as correction for differences in total cranial volume (Blatter et al., 1995).

Using an interactive, atlas-based fitting of outer brain contours derived from single planes in CT or MRI data, a recent study compared male control subjects with both male and female patients who suffered from generalized tonico-clonic seizures (Savic et al., 1998). The contour distortions were explained by an elongation or increase in volume in the frontal lobes and/or atrophy of the cerebellum, the former being consistent with the increase in frontal grey matter in patients with JME described in our study. The methodology of this study was very different from that of our investigation, principally using a mix of MRI and CT data for rater-driven comparisons, selected patients, none of whom was diagnosed as suffering from a specific subsyndrome of IGE, and an entirely male control group.

The differences in the distribution of cortical grey matter voxels demonstrated here between the control subjects and patients with JME may represent differences in the shape of gyri in the mesial pericallosal area rather than reflecting just increases in cortical grey matter volume. Combining the prior knowledge of a widespread increase in grey matter volume in patients with JME (Woermann et al., 1998) with the current finding of voxel-based differences in the distribution of changes between the two homogeneous groups, however, led us to infer increases in grey matter in a mesial pericallosal distribution as being the most likely and dominant mechanism.

Pathophysiological considerations

In epilepsy, structural changes detected on MRI quantitation in the cerebral grey matter may reflect changes in neuronal connectivity (Sisodiya et al., 1995; Woermann et al., 1998). The regional distribution of neocortical grey matter allows inferences to be drawn about the number of cellular elements in these structures, especially those contributing to the neuropil (Caviness et al., 1997). It is not known, however, whether quantitative MRI abnormalities in patients with JME are due to specific anatomical structural changes. Autopsy studies addressing this question are rare in patients with JME. Two out of three patients with JME were shown to have dystopic neurons in the grey matter and other abnormalities (so-called microdysgenesis), and increased neuronal density was reported in the frontal cortex of patients.
with IGE (Janz and Neimanis, 1961; Meencke and Janz, 1984; Meencke, 1985). Structural abnormalities such as microdysgenesis, leading to increases in the volume of the grey matter ribbon, could explain the changes observed in the frontal cortex. However, the definition of microdysgenesis and its meaning in patients with IGE have been disputed (Lyon and Gastaut, 1985). Because there were no estimates of connectivity in terms of numbers of synaptic connections and axonal or dendritic morphology or branching, further histological studies are needed to assess whether structural changes identified on MRI quantitation are due to definite tissue changes. Given the possible diffuse and synaptic nature of changes in patients with JME, histopathological proof, especially of connectivity changes in humans, may not be feasible (Huttenlocher, 1974). Further quantitative MRI, as a surrogate for histology, might enable us to examine the whole of the neocortical and subcortical matter and connectivity in adequate and unbiased detail in vivo (Sisodiya and Free, 1997), but would be strengthened by correlative MRI and pathology studies. Our findings of an increase in grey matter in the mesiofrontal region could guide these studies.

Our findings of abnormal voxel-based MRI quantitation in JME might also suggest a structural correlate of the functional abnormalities in patients with IGE and JME shown recently by EEG studies, neuropsychological testing and functional imaging. Electrophysiological studies have suggested that generalized epileptiform activity in IGE might be generated in the superior frontal cortex (Rodin and Ancheta, 1987; Niedermeyer, 1996). Although some depth EEG studies in humans with IGE found discharges starting in the thalamus before the neocortex (Williams, 1953; Velasco et al., 1989), other studies failed to provide evidence of a primary thalamic onset in patients with generalized spike–wave complexes (Niedermeyer et al., 1969). Animal data and clinical studies in patients with IGE support the view that cortical hyperexcitability has an important part in the pathophysiology of IGE (Gloor, 1995; Niedermeyer, 1996). Patients with JME had a deficit in performance on a task of working memory that was nearly as severe as that of a group with frontal lobe epilepsy with or without obvious structural frontal lobe lesions (Swartz et al., 1994). A PET study investigating the same paradigm showed an association between impaired visual working memory and decreased $^{[18F]}$Fluordeoxyglucose uptake in frontal cortical areas of patients with JME (Swartz et al., 1996). In a recent $^{[11}C$]Flumazenil-PET study, globally increased benzodiazepine receptor density was found in the cerebral neocortex, thalamus and cerebellum of patients with JME, which was consistent with increased neuronal density and possibly microdysgenesis (Koepp et al., 1997). Our findings support the concept that structural cortical changes may be associated with abnormalities in functional connectivity within the mesiofrontal neocortex and between cortical and subcortical structures in patients with JME.

The value of the clinical use of this method in individual patients is not clear. Not all of our individual patients with JME had abnormal brains on grey matter quantitation and voxel-based analysis. The abnormalities identified represented the statistically unequivocal structural differences, others not reaching statistical significance at the rigorous threshold used. After smoothing the normalized grey matter images for the voxel-by-voxel comparison, smoothed areas of abnormality might have swamped smaller areas of abnormality, thus reducing the sensitivity for individual comparisons. A new finding of this study was the result of a group comparison: a mesiofrontal abnormality in a voxel-by-voxel comparison of grey matter between homogeneous groups of JME patients and control subjects. Nevertheless, despite the homogeneity of the clinical syndrome in the patients of this study, we cannot exclude underlying structural heterogeneity within patients with JME as a possible cause of the variability of abnormalities in individual patients. We are currently developing the use of automated grey and white matter segmentation of MRI combined with voxel-by-voxel analyses to explore the clinical and scientific use of automated image processing in large populations of patients with different epilepsy syndromes.

In conclusion, we have found evidence of structural cerebral abnormalities in patients with JME using an objective voxel-based method. At this point, the analysis of structural MRI data using SPM is a useful technique in the scientific investigation of patients with epilepsy. It may also be useful in other neurological and neuropsychiatric diseases, particularly to delineate the extent of structural abnormality, or when no abnormality is evident and when comparisons of structural MRI in homogeneous groups are appropriate.

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Abnormal cerebral structure in JME


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