Electroclinical, MRI and neuropathological study of 10 patients with nodular heterotopia, with surgical outcomes

L. Tassi,1 N. Colombo,2 M. Cossu,1 R. Mai,1 S. Francione,1 G. Lo Russo,1 C. Galli,3 M. Bramerio,3 G. Battaglia,4 R. Garbelli,4 A. Meroni4 and R. Spreafico 4

Summary
We present the results of a retrospective study on 10 patients operated on for intractable epilepsy associated with nodular heterotopia as identified by high resolution MRI. Seven patients had unilateral heterotopia, one patient had symmetric bilateral heterotopia and two patients had asymmetric bilateral heterotopia. By stereo-electroencephalogram (SEEG) (nine patients) interictal activity within nodules was similar in all cases, and ictal activity never started from nodules alone but from the overlying cortex or simultaneously in nodules and cortex. Excellent outcomes (Engel class Ia, 1987) were achieved in the seven patients with unilateral heterotopia, showing that surgery can be highly beneficial in such cases when the epileptogenic zone is carefully located prior to surgery by MRI and particularly SEEG. For the bilateral cases surgical outcomes were Engel IIa (one patient) or Engel IIIa (two patients). Histological/immunohistochemical studies of resected specimens showed that all nodules had similar microscopic organization, even though their extent and location varied markedly. The overlying cortex was dysplastic in nine patients, but of normal thickness. We suggest that nodule formation may be the result of a dual mechanism: (i) failure of a stop signal in the germinal periventricular region leading to cell overproduction; and (ii) early transformation of radial glial cells into astrocytes resulting in defective neuronal migration. The intrinsic interictal epileptiform activity of nodules may be due to an impaired intranodular GABAergic system.

Keywords: epilepsy surgery; immunocytochemistry; periventricular heterotopia; stereo-EEG

Abbreviations: AD = architectural dysplasia; CB = calbindin; CD = cytoarchitectural dysplasia; CR = calretinin; EEG = electroencephalogram; FLAIR = fluid-attenuated inversion recovery; GFAP = glial fibrillary acidic protein; IR = inversion recovery; MAP2 = microtubule-associated protein 2; MCD = malformations of cortical development; PV = parvalbumin; SEEG stereo-electroencephalogram; SCH = subcortical heterotopia; SE = spin echo; SEH = subependymal heterotopia; TSE = turbo spin-echo


Introduction
Heterotopia are malformations of cortical development (MCD) characterized by the presence of apparently normal brain cells in abnormal positions. Three broad categories are recognized: band heterotopia (double cortex), individual misplaced neurons in the white matter (neuronal heterotopia) and nodules of grey matter within the white matter (nodular heterotopia). Barkovich and Kuzniecky (2000) did not include neuronal heterotopia among MCD due to abnormal migration; however, in the recent classification (Barkovich et al., 2001), conditions of abundant neurons in the white matter are again considered as being due to abnormal neuronal migration.

The original classification of heterotopia by Jacob (1936) did not consider neuronal heterotopia but recognized a subependymal nodular form and a laminar (band) form. Today, nodular heterotopia are further divided into: subependymal heterotopia (SEH) (subsuming periventricular nodular

heterotopias), which appears on MRI as nodular subependymal masses having the same signal intensity as cortical grey matter; and subcortical heterotopia (SCH), which appear as irregular clusters of nodules of grey matter within the white matter (Barkovich and Kjos, 1992; Barkovich et al., 2001).

The prevalences of nodular heterotopia in the general population and in patients with epilepsy are unknown. In a large series of adult epileptic patients, Raymond and colleagues reported that about 2% had nodular heterotopia, constituting about 20% of cases with MCD (Raymond et al., 1994b, 1995). In a series of 132 patients with various forms of cortical dysgenesis seen at the Montreal Neurological Institute, 19 (15%) had nodular heterotopia (see Li et al., 1997). Raymond et al. (1994a) reported that 15% of patients with hippocampal sclerosis had associated MCD, most often nodular heterotopia.

Modern high-resolution imaging techniques, such as thin-section volumetric MRI, allow precise definition of the macroscopic morphology and extent of nodular heterotopia. Thus, these conditions can now be diagnosed in vivo and their association with neurological deficits and epilepsy investigated. However, much less is known about the organization and structure of the nodules themselves. Few autopsy cases have been published, and surgical specimens are rare since it is generally considered that epileptic patients with nodular heterotopia do not respond well to surgery (Li et al., 1997). There have been immunolocalization and dye tracing studies (Spreafico et al., 1998; Hannan et al., 1999; Kakita et al., 2002), and also electrophysiological studies using depth electrodes for presurgical assessment (Francione et al., 1994; Kothare et al., 1998); however, much remains to be learnt about the ‘functional’ relationships between nodules and the overlying cortex.

We present a retrospective evaluation of 10 patients with intractable epilepsy and nodular heterotopia on MRI, who were operated on to relieve the epilepsy. Nine underwent presurgical invasive stereo-electroencephalogram (SEEG), and after surgery the specimens were studied using neurohistological and immunolocalization techniques. Presurgical electroclinical and neurological evaluations, and surgical outcomes are also reported.

**Patients and methods**

Of the 372 patients operated on from May 1996 to December 2002 for drug-resistant partial epilepsy at the Claudio Munari Surgery Centre for Epilepsy, Milan, Italy, 10 (2.7%) presented nodular heterotopia on high-resolution MRI and were included in the present study. Two other patients with small subcortical nodules only detected neuropathologically, and not discernible even after re-evaluation of the MR images, were not considered.

The patients underwent surgery only after comprehensive evaluation that, in addition to MRI, comprised neurological examination and history to establish type, age of onset and frequency of seizures, and comprehensive EEG or video-EEG examination with at least one ictal recording to relate ictal EEG events to clinical manifestations. In nine patients the EEG and MRI data were insufficient to unambiguously locate the epileptogenic zone and thus presurgical SEEG was carried out.

**Neuropsychological battery**

The patients were assessed pre- and postoperatively using the following neuropsychological tests: Paired Word Association Test; Word List (primacy, recency); Semantic Fluency (phonemic); Short Tale Test; Token Test; Digit Span; Corsi Span Memory Tests; Rey Figures (copying and memory); Attention Matrices; Trail Making (A and B); Benton’s Line Orientation Test; Raven’s Colour Matrices; and the Beck Depression Scale.

**MRI**

The following sequences were acquired: transverse spin echo (SE) double echo of the entire brain; coronal turbo spin echo (TSE) T2-weighted; coronal TSE fluid-attenuated inversion recovery (FLAIR) T2-weighted; and coronal TSE inversion recovery (IR) T1-weighted. The coronal sequences were acquired around the epileptogenic zone as surmised from the electroclinical data. In most patients, 3D volume fast-field echo T1-weighted images were also obtained. Additional FLAIR or TSE T2-weighted images in the sagittal plane were obtained as necessary. In patients suspected of having temporal lobe epilepsy, transverse images were acquired parallel, and coronal images acquired perpendicular, to the major hippocampal axis. For extratemporal lobe epilepsies, slices were acquired parallel and perpendicular to the bicommissural line.

The MR images were assessed by two neuroradiologists independently with the aim of identifying: nodules in the subcortical white matter or subependymal region; cortical gyration anomalies; focal thickenings of the cortex (assessed by comparison of affected site with corresponding site contralaterally and in aged-matched normal controls); abnormal signal intensity in the cortex and subcortical white matter; blurring of the grey–white matter junction; and abnormalities of corpus callosum, cerebellum, ventricular system and basal ganglia. Evidence of hippocampal sclerosis was also sought as hippocampal atrophy, increased signal on T2-weighted images, decreased signal on T1-weighted images and loss of definition of internal structures.

**SEEG**

Stereo arteriography to localize major blood vessels was performed first. Some weeks later multilead (5–18) electrodes (Dixi, Besançon, France) were implanted intracerebrally under general anaesthesia using a technique similar to that described by Talairach and Bâncaud (1966). After implantation, recording took place for 5–10 days under clinical and video control; at least one seizure was recorded. A few days after electrode implantation, 3D MRI was performed to verify electrode trajectory and location in relation to the nodular heterotopia and suspected epileptogenic zone. During SEEG, intermittent light stimulation (1–30 Hz) and low (1 Hz) and high (50 Hz) frequency electrical stimulation were performed in order to identify sensory and motor cortical areas and induce ictal phenomena. The SEEG data were assessed by at least two neurologists. The 3D reconstructions produced from the SEEG data were compared with the lesions revealed by MRI, paying particular attention to the information obtained from the electrodes probing nodules and the cortex overlying the nodules. When recordings sufficiently informative for planning surgery had been obtained (epileptogenic areas identified), the electrodes were removed.
Surgery

Surgery was performed only after informed consent was given by the patient, or the parents of intellectually impaired individuals. The aim of surgery was strictly therapeutic: to remove accessible brain areas involved in seizure generation. The patient’s neurological condition and likelihood of postsurgical neurological deficits were also considered in determining the final surgical approach.

In the patients who underwent SEEG, electrode tracks in the resected specimens were identified visually by the surgeon and served to provide spatial correlations between the electrical, MRI and neuropathological data.

Histology and immunohistology

Specimen preparation procedures are described in detail elsewhere (Spreafico et al., 1998; Tassi et al., 2001). For routine neuropathology, specimens were fixed in 10% neutral buffered formalin embedded in paraffin, and 4- to 10-mm thick sections were cut and stained using routine methods that included thionin and Luxol Fast Blue staining. Additional series of 4-mm thick serial sections were cut and processed for immunolocalization. Fifty micrometre vibratome sections were also cut and processed for immunocytochemistry (Spreafico et al., 1998, 2000). These thicker sections provided additional visualization of immunoreactivity, which served as a check on the thin section findings. After incubation in 10% (v/v) normal serum to mask non-specific adsorption sites, sections were processed using primary antibodies to the following antigens: glial fibrillary acidic protein (GFAP; (Boehringer Mannheim, Germany) as marker of astrocytes, the intermediate filament protein vimentin (VIM; Dako, Denmark), SMI 311 (Sternberger Monoclonal, Denmark) as marker of non-phosphorylated neurofilaments, microtubule-associated protein 2 (MAP2) (Boehringer Mannheim), and the calcium-binding proteins (Swant, Swiss) parvalbumin (PV), calbindin (CB) and calretinin (CR). The characteristics and working dilutions of the antibodies used are listed in Table 1.

The sections were incubated for 24 h in primary antibody, rinsed in Tris-buffered saline (TBS), and incubated for 1 h in biotinylated secondary antibody (goat anti-rabbit or horse anti-mouse IgGs, depending on the primary) diluted 1 : 200 in phosphate-buffered saline (PBS). The sections were then rinsed in TBS, and incubated for 2 h in avidin-biotinylated complex (ABC kit; Vector Inc., Burlingame, CA, USA), diluted 1 : 100 in PBS. After rinsing in TBS and 0.05 M Tris–HCl buffer (pH 7.6), sections were reacted with a freshly prepared solution (0.075%) of 3,3'-diaminobenzidine tetrahydrochloride (Sigma) and 0.002% H₂O₂ in Tris–HCl for 5–10 min, washed in Tris–HCl, dehydrated and covered. Adjacent sections were stained with 0.1% thionin for structural control.

Results

The principal characteristics of the 10 patients (five male, five female) are shown in Table 2. Mean age at surgery

### Table 1 Type, specificity and dilutions of primary antibodies used

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Dilution</th>
<th>Type</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-VIM</td>
<td>1 : 3000</td>
<td>Mouse monoclonal IgG</td>
<td>Intermediate filaments in radial glia, and reactive astrocytes</td>
</tr>
<tr>
<td>Anti-GFAP</td>
<td>1 : 500</td>
<td>Mouse monoclonal IgG</td>
<td>Intermediate filaments in astrocytes and reactive astrocytes</td>
</tr>
<tr>
<td>Anti-SMI 311</td>
<td>1 : 1000</td>
<td>Mouse monoclonal IgG</td>
<td>Non-phosphorylated neurofilaments</td>
</tr>
<tr>
<td>Anti-MAP2</td>
<td>1 : 200</td>
<td>Mouse monoclonal IgG</td>
<td>Microtubule associated proteins 2a and b</td>
</tr>
<tr>
<td>Anti-PV</td>
<td>1 : 10 000</td>
<td>Mouse monoclonal IgG</td>
<td>Subpopulations of GABAergic interneurons</td>
</tr>
<tr>
<td>Anti-CB</td>
<td>1 : 10 000</td>
<td>Mouse monoclonal IgG</td>
<td>Subpopulations of GABAergic interneurons</td>
</tr>
<tr>
<td>Anti-CR</td>
<td>1 : 5000</td>
<td>Mouse monoclonal IgG</td>
<td>Subpopulations of GABAergic interneurons</td>
</tr>
</tbody>
</table>

### Table 2 Main clinical characteristics of the 10 patients

<table>
<thead>
<tr>
<th>Patient and sex</th>
<th>Family antecedents</th>
<th>Personal antecedents</th>
<th>Febrile convulsions</th>
<th>Neurologic examination</th>
<th>Age at seizure onset (years)</th>
<th>Duration of epilepsy (years)</th>
<th>Monthly seizure frequency</th>
<th>SEEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M</td>
<td>No</td>
<td>&lt;2 kg when born (8th month)</td>
<td>No</td>
<td>Normal</td>
<td>16</td>
<td>23</td>
<td>4</td>
<td>No</td>
</tr>
<tr>
<td>2 F</td>
<td>No</td>
<td>Twin; sister died during dystocial delivery</td>
<td>No</td>
<td>Normal</td>
<td>15</td>
<td>23</td>
<td>8</td>
<td>Yes</td>
</tr>
<tr>
<td>3 F</td>
<td>Uncle and cousin with unspecified epilepsy</td>
<td>Risk of spontaneous abortion at 3rd month</td>
<td>No</td>
<td>Normal</td>
<td>14</td>
<td>19</td>
<td>15</td>
<td>Yes</td>
</tr>
<tr>
<td>4 F</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>9</td>
<td>15</td>
<td>6</td>
<td>Yes</td>
</tr>
<tr>
<td>5 F</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>11</td>
<td>9</td>
<td>2</td>
<td>Yes</td>
</tr>
<tr>
<td>6 M</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>17</td>
<td>10</td>
<td>30</td>
<td>Yes</td>
</tr>
<tr>
<td>7 M</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>16</td>
<td>8</td>
<td>10</td>
<td>Yes</td>
</tr>
<tr>
<td>8 F</td>
<td>No</td>
<td>Neonatal respiratory distress and cyanosis</td>
<td>No</td>
<td>Normal</td>
<td>3</td>
<td>37</td>
<td>20</td>
<td>Yes</td>
</tr>
<tr>
<td>9 M</td>
<td>No</td>
<td>No</td>
<td>Abnormal</td>
<td>No</td>
<td>6</td>
<td>33</td>
<td>25</td>
<td>Yes</td>
</tr>
<tr>
<td>10 M</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>2</td>
<td>19</td>
<td>50</td>
<td>Yes</td>
</tr>
</tbody>
</table>

M = male; F = female.
was 31 years (range 20–40; SD 8) and mean duration of epilepsy was 20 years (range 8–37; SD 10). Mean age at epilepsy onset was 11 years (range 2–17; SD 6) and mean seizure frequency at surgery was 17 per month (range 2–50; SD 15). Patients 8, 9 and 10, with bilateral heterotopia on MRI (see below), had intelligence quotients <80. In patient 10 the heterotopia was more evident on the left and he also had mild left hemiparesis. Patient 1 had typical temporal lobe seizures with epileptiform discharges originating in the right temporal lobe. MRI revealed small nodular formations in the right temporal lobe. We considered this information a sufficient guide to surgery and SEEG was not performed.

**MRI**

The MRI findings are summarized in Table 3. Nodules were unilateral in patients 1–7 (on the right in six). In five of these unilateral cases the nodules were isointense with normal grey matter, and in two were moderately hyperintense on T2-weighted FLAIR sequences.

In patient 3, with left heterotopia, a small cluster of nodules was observed in the left peririgional region (Fig. 1A and B). In the other patients multiple nodules were present in the right temporo-occipital region (Fig. 1D and E); in two cases they were exclusively subependymal (SEH) and in four they were present in the white matter from the ventricular wall to close to the cortex (SCH).

In all seven unilateral cases, scattered radial bands, of the same signal intensity as grey matter, extended from the nodules to the cortex (Fig. 1B, arrowhead); the overlying cortex was abnormal, usually polygyric, without signal alterations; cortical thickness was reduced in patients 4 and 5 and was normal in the others. In five (patients 1, 2 and 4–6) of the six patients with extensive temporo-occipital nodules, the involved lobes were of small size compared with the contralateral side, and in five cases (patients 2 and 4–7) the ventricle was distorted by the presence of the nodules.

In none of the seven unilateral cases were white matter signal abnormalities, basal ganglia distortion or corpus callosum alterations observed. Mega cisterna magna was present in patient 4. In patients 2 and 4, the hippocampus ipsilateral to the nodular formation was incompletely rotated, suggesting a dysembryogenetic alteration, but without signs of hippocampal sclerosis.

Three patients had bilateral nodules. Patient 8 had symmetric bilateral involvement, with periventricular nodular bands of heterotopia extending along the entire contour of the ventricles, moderately hyperintense in T2-weighted FLAIR images, which did not indent the ventricle profiles. In addition a tiny band of grey matter, isointense with the cortex, was discerned between the nodules and the outer cortex, in the frontal lobe on the left, and in the temporal and occipital lobes on both sides (Fig. 1G–I) (incomplete, asymmetric double cortex). Mild disseminated cortical atrophy was also evident, but signal alterations in the cortical mantle were absent. No abnormalities in the corpus callosum, basal ganglia, cerebellum or hippocampus were observed in this patient.

In the other patients with bilateral involvement (patients 9 and 10), the heterotopia was markedly asymmetric and the large nodular formations indented the ventricles, and extended towards the cortex. The most affected hemisphere was hypoplastic relative to contralateral hemisphere (Fig. 1J and K). Nodular signal intensity was the same as that of the overlying cortex. In patient 9 the basal ganglia were deformed, the corpus callosum reduced in size, the cisterna magna enlarged and the ipsilateral hippocampus rounded suggesting incomplete rotation, although the hippocampal signal was normal. In patient 10 mega cisterna magna and left cerebellar hypoplasia were present, but no other non-heterotopic abnormalities were observed.

**SEEG**

In patient 8, none of the implanted electrodes made contact with a nodule. In the eight remaining patients more than one electrode made contact with nodules, and at least two recording contacts were located in different regions of the nodular formation. In these eight patients, the electrodes within the temporal lobe simultaneously explored the neocortex, the nodular formations and mesial structures (amygdala and hippocampus).

**Intercital activity**

Awake and asleep interictal activity was recorded in all eight patients. While awake, background activity from nodules was low voltage low frequency interrupted by frequent high voltage spikes followed by positive waves. These spike and wave complexes (Fig. 2) came only from nodules and were similar in all eight patients irrespective of where the probed nodules were situated. We have never observed similar SEEG activity in patients with other lesions or cortical malformations (specifically focal cortical dysplasia, polymicrogyria and tuberous sclerosis).

In patients where recordings were obtained from more than one nodule, generally one nodule showed a more sustained almost pseudorhythmic interictal activity, and interictal spikes were always asynchronous between different nodules (Fig. 3A). Background activity from hippocampal regions (in the six patients with electrodes in mesial regions of the temporal lobe) and from neocortex overlying nodules was either normal or slowed, except that voltages were higher than those recorded from nodules. Interictal spikes from nodules were often synchronous with spikes, or spikes and waves from overlying cortex, but were seldom synchronous with activity from mesial temporal structures (Fig. 2A and B). During intermittent light stimulation, photic recruitment was observed in the temporal-occipital regions in all eight cases, but only in three was recruitment synchronous in nodules and overlying cortex for frequencies 1–20 Hz (Fig. 3B).
Table 3  Summary of MRI findings

<table>
<thead>
<tr>
<th>Patients</th>
<th>Type</th>
<th>Side</th>
<th>Site</th>
<th>Signal intensity (nodules versus cortex)</th>
<th>Cortex thickness</th>
<th>Gyrations</th>
<th>Hemisphere size</th>
<th>Ventricular distortion</th>
<th>WM signal alteration</th>
<th>Basal ganglia</th>
<th>Corpus callosum</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SEH</td>
<td>Unilateral</td>
<td>Right</td>
<td>Temporal and peritrigonal</td>
<td>Iso</td>
<td>Normal</td>
<td>Altered</td>
<td>Reduced temporal pole</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>SCH</td>
<td>Unilateral</td>
<td>Right</td>
<td>Temporal-occipital</td>
<td>Hyper in T2-FLAIR</td>
<td>Normal</td>
<td>Altered</td>
<td>Reduced temporal-occipital</td>
<td>Yes</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>3</td>
<td>SEH</td>
<td>Unilateral</td>
<td>Left</td>
<td>Peritrigonal</td>
<td>Iso</td>
<td>Normal</td>
<td>Altered</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>4</td>
<td>SCH</td>
<td>Unilateral</td>
<td>Right</td>
<td>Temporal-occipital</td>
<td>Iso</td>
<td>Reducer temporo-basal</td>
<td>Altered</td>
<td>Reduced temporal pole</td>
<td>Yes</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>5</td>
<td>SCH</td>
<td>Unilateral</td>
<td>Right</td>
<td>Temporal-occipital</td>
<td>Iso</td>
<td>Reduced temporal-occipital</td>
<td>Altered</td>
<td>Reduced temporal-occipital</td>
<td>Yes</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>6</td>
<td>SCH</td>
<td>Unilateral</td>
<td>Right</td>
<td>Temporal-occipital</td>
<td>Hyper in T2-FLAIR</td>
<td>Normal</td>
<td>Altered</td>
<td>Reduced temporal-occipital</td>
<td>Yes</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>7</td>
<td>SEH</td>
<td>Unilateral</td>
<td>Right</td>
<td>Temporal-occipital</td>
<td>Iso</td>
<td>Normal</td>
<td>Altered</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>8</td>
<td>SEH</td>
<td>Bilateral</td>
<td>Symmetric</td>
<td>–</td>
<td>Hyper in T2-FLAIR</td>
<td>Diffusely reduced</td>
<td>Normal</td>
<td>Normal</td>
<td>No</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>9</td>
<td>SCH</td>
<td>Bilateral</td>
<td>Asymmetric</td>
<td>–</td>
<td>Iso</td>
<td>Reduced right side</td>
<td>Altered bilaterally</td>
<td>Reduced right hemisphere</td>
<td>Yes (right)</td>
<td>No</td>
<td>Distorted right side</td>
<td>Reduced</td>
</tr>
<tr>
<td>10</td>
<td>SCH</td>
<td>Bilateral</td>
<td>Asymmetric</td>
<td>–</td>
<td>Iso</td>
<td>Reduced right side</td>
<td>Altered bilaterally</td>
<td>Reduced left temporal lobe and right occipital lobe</td>
<td>Yes (left)</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Iso = Isointense.
Fig. 1 MRI. (A) Sagittal and (B) coronal TSE IR T1-weighted images (3000/20/400/2) from patient 3, showing multiple nodules in left peritrigonal subependymal region (arrows). The nodules have the same signal intensity as the overlying grey matter. Radial bands (arrowhead) connect the nodule with the overlying occipital cortex showing an abnormal gyration. C is a coronal TSE IR T1-weighted postsurgery image (3000/20/400/2) from the same patient showing that the nodular formation and occipital neocortex have been removed. D and E are axial TSE IR T1-weighted images (3000/20/400/2) at two different levels from patient 5 displaying massive unilateral subcortical heterotopia in the right temporal-occipital lobes (arrows), extending from the ependymal surface of the ventricle toward the immediate subcortical region and also involving the cortex. The neocortex is thin and multigyrlic in D and thickened in E. F is an axial TSE IR T1-weighted postsurgery image (3000/20/400/2) from the same patient showing a large surgical cavity with residual nodular heterotopia along the posterior cavity boundary (arrow). G and H are coronal TSE IR T1-weighted images (3000/20/400/2) from patient 8 with bilateral subependymal heterotopia. In G a thin band of grey matter is visible within the subcortical white matter of the left frontal lobe (arrowheads and enlarged inset), representing an incomplete double cortex. In G and H symmetrical periventricular nodules are visible along the walls of the lateral ventricles (arrows). I is a coronal TSE IR T1-weighted postsurgery image (3000/20/400/2) in the same patient at the same level as G, showing that the left frontal neocortex has been removed. This was identified as the epileptogenic zone by SEEG; the nodular heterotopia was not removed. J is an axial TSE IR T1-weighted image (3000/20/400/2) of patient 10 showing extensive subependymal nodular heterotopia in left temporal-occipital region, extending toward the cortical mantle. Note abnormal gyrations, thickened cortex and the ventricle indented by the malformation. K is a coronal TSE IR T1-weighted image (3000/20/400/2) of the same patient showing subependymal heterotopic nodules in the right temporo-occipital region. Cerebellar hypoplasia with mega cisterna magna is also evident. L is an axial TSE IR T1-weighted postsurgery image (3000/20/400/2) of the same patient, at the same level as J, showing the huge surgical cavity. Superficial and deep parts of the left temporal-occipital lobes were removed.
Ictal activity
Ictal activity (fast, low voltage) was recorded in all nine patients. In the eight patients with electrodes contacting nodules and overlying cortex, 77 seizures were recorded (mean 9.6 per patient). In no case did ictal activity clearly start from the nodules (Fig. 4A), but in five cases simultaneous activation of one nodule and the overlying cortex was observed (Fig. 4B). In the remaining three patients, ictal discharges originated from the cortex.

In two of the six patients with hippocampal electrodes, ictal discharges in recorded nodules and hippocampus were synchronous, but in no case did ictal activity clearly start from the mesial regions of the temporal lobe. Neither low nor high frequency stimulation of the nodules ever induced seizures.

Surgery and outcome
Corticectomy was performed in all patients. A frontal resection was performed in patient 8, a parieto-occipital resection was performed in patient 10 and temporal resections, with part of the hippocampus also removed, were performed in eight patients. Part of the heterotopic nodular formation was removed in eight patients, while in patient 3, with a small left peririgonal nodular formation, a temporal-basal corticectomy was performed with total removal of the nodular formation (Fig. 1C). In patient 8, with symmetrical bilateral nodular heterotopia, a left frontal corticectomy was performed without ablation of the periventricular nodular formation (Fig. 1I). The right temporal lobe only was the intervention site in four patients (1, 2, 4 and 6). In two patients (Fig. 1C and F), parts of the temporal and
occipital lobes were ablated (patient 3 left side; patient 5 right side) and in three patients parts of the temporal, parietal and occipital lobes were removed (patients 7 and 9 right side; patient 10 left side; Fig. 1L). The hippocampus was resected in eight patients. There was no postsurgical mortality. In none of the patients did neuropsychological test scores worsen after surgery. Hemianopsia developed, contralateral to the side of surgery, in two patients, and quadrantopsia in six. All patients were advised of the high probability of visual field deficits prior to surgery.

Fig. 3 Interictal SEEG recordings. (A) Patient 2: different nodules (labelled ‘Heterotopia’) show different and asynchronous interictal patterns. (B) In the same patient: intermittent photic stimulation (9 Hz) induces recruitment of lingual gyrus, inferior occipital gyrus, cuneus and postcentral gyrus, and in one of the heterotopic nodules. Lat = lateral; T = temporal; Ant Hippo = anterior hippocampus; Post Hippo = posterior hippocampus; g = gyrus; Inf = inferior; O = occipital; Supram = supramarginal; Sup = superior; P = parietal; Lob = lobe; C = central; F = frontal; Postcentr = postcentral.
All patients have a follow-up of at least 1 year. Seven patients have an Engel class Ia (Engel, 1987) outcome, one has a class IIa outcome and the remaining two have class IIIa outcomes. Surgical characteristics, follow-up and outcomes are summarized in Table 4.

**Histological and immunohistological findings**

**Cortex**

In all patients the thickness of the resected cortex appeared normal with cell density also apparently normal.

---

Fig. 4 Ictal SEEG recordings. (A) Spontaneous seizure recorded in patient 2, showing secondary involvement of some nodules and no involvement of others. (B) Spontaneous seizure in patient 6 illustrates simultaneous involvement of nodules and explored lateral cortex. Sup = superior; T = temporal, g = gyrus; Mes = mesial; Lat = lateral; Ant Hippo = anterior hippocampus; Post Hippo = posterior hippocampus; Inf = inferior; Supram = supramarginal; P = parietal; Mid = middle; O = occipital; Inf = inferior.
In one patient (patient 3, with unilateral temporo-occipital heterotopia) the specimen showed moderate neocortical and hippocampal gliosis, while laminar organization was normal and no cytological abnormalities were discerned (Table 4). In the other nine patients the cortex was dysplastic. We classified the dysplasia according to Tassi et al. (2002). Patients 1, 2, 4–7 and 9 (six with unilateral and patient 9 with bilateral asymmetric heterotopia) had architectural dysplasia (AD) and two (patients 8 and 10) had cytoarchitectural dysplasia (CD). In the AD cases layers I and II were usually clearly evident, but the border between layers III–VI was often unrecognizable; in patients 4 and 6 layer IV was completely absent. No cytological abnormalities were observed (Fig. 5A and B).

In patient 1, with AD, a small glioneuronal hamartoma was found. In patients 5 and 10, the gyri were abnormally numerous (Figs 5G and 6A); however, the microscopical features of the grey matter did not resemble those observed in unlayered or four-layered polymicrogyric cortex (Barth, 1987; Ferrer and Català, 1991). In most of the cortical specimens areas of dysplastic cortex were intermingled with areas of normally layered cortex.

The immunolocalization studies supported the diagnosis of AD in all seven cases, showing reduced immunoreactivity for SMI311-, MAP2- (Fig. 5B) and PV-reactive cells and terminals (Fig. 5C). Use of VIM antibody did not reveal the presence of radial glial fibres in any specimen.

Hippocampal material was removed from all eight patients with temporally located nodules; however, neuropathological examination of the hippocampus was only possible in seven cases: in patient 4 the tissue was too fragmented to permit hippocampal diagnosis. In patients 2 and 6 hippocampal structures were normal; in patients 1, 3, 5, 7 and 9 gliosis of varying severity, as revealed by GFAP immunoreactivity, was observed, but in none were typical signs of hippocampal sclerosis (cell loss in the endfolium and regions 1 and 3 of Ammon’s horn, or dispersion granular cells) observed (Table 4). In the hippocampal formation specimens analysed, neither conformational nor dysembryogenic alterations were revealed, as suspected by MRI.

In patient 8, with bilateral symmetric heterotopia, and patient 10, with bilateral asymmetric heterotopia, CD was diagnosed on the basis of the presence of giant neurons throughout the cortex (Fig. 6C, D and F) in addition to disruption of the cortical layering (Fig. 6E). It is noteworthy that patients 8 and 10 were the only ones not to be operated on in the temporal region. In patient 10 the cortex overlying the nodules was particularly disrupted by the presence of heterotopic nodules invading the cortical mantle (Fig. 6H–J); excessively numerous gyri were evident, but, as with patient 5 (Fig. 5) no cytological signs of polymicrogyric cortex were detected (Fig. 6A).

In specimens from all patients, numerous heterotopic neurons were present in the subcortical white matter and were more numerous in the vicinity of the nodules. These neurons frequently formed clusters and were particularly evident in sections processed to reveal MAP2. Small numbers of these heterotopic neurons were SMI311- or CR-positive, but none was PV- or CB-positive. Some of the MAP2- and SMI311-positive heterotopic neurons had the morphology of small pyramidal cells, while the small cells immunoreactive for CR were rounded or fusiform. Moderate to severe gliosis of the grey and white matter was revealed by GFAP immunocytochemistry, but severity seemed unrelated to dysplasia type.

### Nodules

Heterotopic nodules were present in resected material from all but one of the removed specimens (that from patient 3). In sections processed for routine staining the nodules were of variable size and formed by aggregates of nerve cell bodies interspersed with bundles of myelinated fibres (Fig. 6B). With Luxol Fast Blue, small bundles of fibres were observed to penetrate the nodules or to cross the border between nodule and white matter and were present within nodules (Fig. 5D).

Moderate gliosis was always present within nodules as revealed by the presence of GFAP-positive cells, particularly around blood vessels. Irrespective of size, lobe location or depth location (close to the cortex or confined to ependymal...
Fig. 5 Photomicrographs of sections from specimens of patient 5 with unilateral nodular heterotopia. A and B are thionin stained and MAP2 immunostained, respectively, low-power photomicrographs of dysplastic temporal cortex overlying the nodular formation. Calibration bar = 200 μm. Note in A the columnar arrangement of the cortex, while MAP2 immunostaining in B reveals the cortical disorganization. C is a PV-immunostained section of cortex. Note the reduction in interneurons compared to the normal temporal cortex in C′ (magnification similar to C). Calibration bar = 200 μm. D is a Luxol Fast Blue-stained section showing bundles of fibres (arrows) within a heterotopic nodule. Calibration bar = 200 μm. E and F are serial sections of the same nodule as in D immunostained to reveal MAP2 and PV, respectively, and showing pyramidal neurons and interneurons. Note in F the presence of areas devoid of PV-positive interneurons (asterisks) and other areas with clusters of immunoreactive cells. Calibration bars = 200 μm. wm = white matter. G is a photomicrograph of multigyr cortex with NeuN immunostaining. Multigyria is frequently observed overlying nodules. Calibration bar = 800 μm. H is a high-power photomicrograph of PV-immunoreactive interneurons within a nodule showing chandelier cells. Calibration bar = 20 μm. I is a high-power photomicrograph of a cluster of MAP2-immunostained neurons within a nodule. Note that despite their pyramidal morphology only a thin rim of labelled cytoplasm surrounds large unstained nuclei, suggesting incomplete maturation. Calibration bar = 20 μm.
Fig. 6 Photomicrographs derived from specimens of patient 10 with asymmetric bilateral nodular heterotopia as shown in Fig. 1J–L. A is a low-power photomicrograph of a Luxol Fast Blue-stained section of occipital cortex, white matter, subependymal region and ventricle. Note the nodular formations around the ventricle (asterisk) and overlying cortex. Calibration bar = 0.5 cm. B is a high-power detail of one of the nodules in A (square), showing myelinated fibres. Calibration bar = 20 μm. C and D are high-power photomicrographs of PV-positive interneurons and large SMI311-positive neurons, respectively, in a heterotopic nodule from the occipital periventricular area. Calibration bars = 20 μm. E, F and G are low-power photomicrographs of serial sections of dysplastic occipital cortex overlying nodules stained with thionin (E) and immunostained to reveal neurofilaments (F) and PV (G). Giant pyramidal neurons are discernible in F (arrows) permitting diagnosis of cytoarchitectural dysplasia. PV-positive cells (G) are reduced in number compared with normal cortex (Fig. 4C’). Calibration bars = 200 μm. H, I and J are sections from occipital cortex stained with thionin (H) and immunostained to reveal MAP2 (I) and PV (J). In this patient the heterotopic nodules reach the cortex resulting in complete disruption of cortical organization, evident by comparison with E, F and G. Clusters of neurons immunostained with MAP2 and PV are mingled with unstained fibres. Calibration bars = 200 μm. K, L, M and N are low-power photomicrographs of serial sections of the border of a nodule stained with thionin, anti-SMI311, anti-MAP2 and anti-PV, respectively. Although the organization of the nodules is similar to that observed in other patients, in this case giant SMI311-positive neurons (arrows L) similar to those in the overlying cortex are observed (see F). Note also scattered MAP2-positive neurons (M) in the white matter (wm) adjacent to the nodules. Calibration bars = 200 μm.
region), the structural organization of the nodules was always similar; laminar organization was never observed. Nodule borders were characterized by the presence of small MAP2-positive fusiform bipolar cells (Figs 5E and 6M), whose proximal dendrites were seen, at high magnification, to run parallel to the fibre bundles surrounding the nodules.

Small and medium-sized SMI311- and MAP2-positive neurons were present within nodules, frequently arranged in clusters (Fig. 5E). Most of these neurons were pyramidal in shape with haphazardly oriented apical dendrites, large nucleus and a thin rim of cytoplasm, suggesting incomplete differentiation (Fig. 5I). Intermingled with these neurons were cells and terminals immunoreactive for CB and CR, as ascertained in serial sections. In sections processed to reveal PV, clusters of immunopositive neurons and labelled puncta, interpreted as terminals or cross sections of dendrites and axons, were present intermingled with areas of unlabelled neuropile (Figs 5F and 6N). These neurons (Fig. 5H) had the same morphology and shape as the PV-positive chandelier and basket interneurons cells in the overlying neocortex. Although counting was not performed, cells positive for calcium-binding proteins were particularly numerous within nodules.

In patient 10, with bilateral asymmetrical nodular heterotopia presenting cortical CD, enlarged pyramidal neurons heavily positive for SMI311 were present in nodules as well as the overlying cortex (Fig. 6D). In this patient, PV-positive neurons were also large (Fig. 6C), compared with PV-positive cells in the cortex, and in nodules from the other nine patients. Thus, in all cases, nodule cytology was similar to that of the overlying cortex.

Discussion

Thanks to increased understanding of the mechanisms, including genetic mechanisms, of cortical development, and improvements in in vivo diagnosis by high-resolution MRI, nodular heterotopia and other malformations of cortical development have become objects of renewed scientific and clinical interest. An X-linked dominant inheritance has been established for familial bilateral periventricular nodular heterotopia (PNH) in females, a condition also characterized by a high incidence of spontaneous miscarriages particularly in male fetuses. Linkage analysis mapped this disorder to Xq28 (Huttenlocher et al., 1994; Dobyns et al., 1996; Eksioğlu et al., 1996; Fink et al., 1997). Subsequently the gene responsible, filamin 1 (FLN1), was identified.

Sporadic cases of bilateral periventricular nodular heterotopia both in females and males have also been described (Sisodiya et al., 1999; Sheen et al., 2001; Kakita et al., 2002). Nodular heterotopia are also observed in other conditions for with multiple causative genes and environmental aetiologies are suggested (Barkovich and Kjos, 1992; Battaglia et al., 1997; Sheen and Walsh, 2003).

Genetic studies are in progress in the patients from the present series. However, the clinical presentations, family histories and imaging data suggest aetiologies unrelated to FLN1 mutations. The present report is concerned with the electroclinical, neuroimaging and pathological characteristics, as well as surgical outcomes, in this consecutive series of patients with MRI-documented nodular heterotopia operated on for intractable epilepsy. The only other surgical series of comparable size to be published is that of Li et al. (1997).

Based on MRI features, we were able to divide the patients in three groups. The first consisted of seven patients (patients 1–7) with unilateral nodular heterotopia, mainly on the right, as also reported by Raymond et al. (1994b). In this group seizure onset was generally later than in patients with other MCD/epilepsy syndromes, in agreement with Barkovich and Kuzniecky (2000). Furthermore, no neurological, behavioural or psychological deficits were evident, and the electroclinical data were insufficiently informative, except in one case, to identify the epileptogenic zone. For this reason we had to use SEEG. Our MRI findings also allowed division of this group into three with SEH and four with SCH in accordance with the currently accepted classification (Barkovich and Kuzniecky, 2000; Barkovich et al., 2001). However at variance with this classification: (i) none of our patients had agenesis or hypogenesis of the corpus callosum; (ii) malformations in the cerebellum, basal ganglia and thalamus were generally absent (one patient only had a mega cisterna magna); and (iii) only two of the patients with SCH had a thin overlying cortex, although in three of the four hemispheric size was reduced.

The second group consisted of a single patient with bilateral symmetrical SEH (patient 8). The nodular formations in this patient were hyperintense relative to the cortex, and cortical thickness was reduced everywhere. The case was unusual for the presence of subtle bands of malformation within the white matter (incomplete double cortex) having the same signal intensity as the overlying cortex. To our knowledge no case such as this has been described previously. The clinical presentation of this patient differed from that in the first group, being characterized by early seizure onset (3 years) and mild mental retardation.

The third group (patients 9 and 10) had bilateral asymmetrical SCH associated with other cortical/brain malformations; patient 9 also had neurological and behavioural deficits. Consistent with the proportions in this series, SCH is reported as less common than SEH (Barkovich and Kuzniecky, 2000). In their study on 33 patients with nodular heterotopia, Dubeau et al. (1995) described 10 patients with SCH; however, all were unilateral. In the study of Battaglia et al. (1997) only two of the seven patients with bilateral PNH had asymmetric SCH.

The relation of hippocampal sclerosis to nodular heterotopia is unclear. Raymond and colleagues reported that it was associated with various types of cortical dysgenesis, most commonly with nodular heterotopia (Raymond et al., 1994a, b). Six of the 10 nodular heterotopia patients described by Li et al. (1997) had hippocampal sclerosis. In our series, three patients had abnormalities suggesting hippocampal
dysgenesis, but none had MRI or pathological signs of hippocampal sclerosis. A novel finding of our MRI study was that, in three patients, T2 FLAIR sequences revealed heterotopic nodules that were slightly more intense than the overlying cortex. In these patients the nodules were isointense by standard T2 and SE T2 sequences, in agreement with the literature (Barkovich et al., 2000; Dubeau et al., 1995; Raymond et al., 1995). To our knowledge, no other studies have used T2 FLAIR sequences to investigate heterotopia. In two of these patients, pathological data on the nodules were available and findings were indistinguishable from those of other nodules.

**SEEG**

Sisodiya (2000) noted that EEG-based methods and neuroimaging promised to provide improved means of identifying the epileptogenic zone or malformation prior to resection. However, scalp EEGs in epileptic patients with MCD often show widespread or multifocal interictal spiking, and ictal onset is generally difficult to localize (Raymond and Fish, 1996). Only in one of our patients did non-invasive methods provide sufficient information to determine the resection site. In this patient the electroclinical and MRI data were consistent with temporal lobe seizure origin allowing tailored surgery without SEEG. In the other patients clear correlations between seizure characteristics, ictal EEG recordings and extent of anatomical malformation were not found. SEEG seems to be the best currently available tool for pinpointing the epileptogenic zone and for investigating electrical interactions between the cortex and underlying heterotopic structures (Francione et al., 1994; Mai et al., 2003), even though its utility is limited by the small volume explored by each electrode (Munari and Bancaud, 1985; Munari et al., 1994; Chassoux et al., 2000; Tassi et al., 2001).

SEEG allowed us to limit the surgery to removal of the epileptogenic zone: in most patients we leave part of the nodular malformation in place; only in patients 1 and 3 were all nodules removed. Electrocorticography and subdural electrodes are of little use since the electrodes do not contact the heterotopic areas.

Li et al. (1997) used depth electrodes to assess six patients with nodular heterotopia but no electrodes reached the heterotopic nodules. Kothare et al. (1998) used depth electrodes to record from nodules in three patients; in two the nodules were apparently the sole source of discharges, while in the other patient only some seizures originated in the heterotopia; unfortunately no electrodes probed the overlying cortex. In the case of nodular heterotopia studied by Francione et al. (1994) using SEEG, electrical anomalies were present within the heterotopia and cortex and the seizures originated simultaneously from both. In the present series, using the same methodology, we never recorded seizure onset from the explored nodules: among the eight patients in whom both cortex and nodules were investigated, ictal electrical activity was recorded simultaneously from cortex and explored nodules in five and a clear cortical origin was detected in the remaining three. Furthermore, electrical stimulation of the nodules did not trigger seizures or other phenomena interpreted by the patient as the onset of a seizure. In contrast, electrical stimulation of the cortex triggered subjective phenomena recognized by the patients as the initial stages of seizures. These findings indicate that the cortex was the principal point of origin of the seizures. Furthermore, outer cortex and nodules were activated simultaneously (in five patients), suggesting that nodules and overlying cortex were anatomically and functionally interconnected. This hypothesis is supported by recent electro-physiological findings (Chen et al., 2000) on Tish rats, which showed that seizure activity began almost simultaneously in normotopic and heterotopic areas, and that blocking communication between the two cortices inhibited spiking in the heterotopic but not normotopic outer cortex.

Another interesting finding of the present study concerned the intrinsic interictal activity within nodules, characterized by frequent high voltage spikes followed by positive waves (Fig. 2). These characteristics are unlike those obtained from invasive recording of other kinds of cortical malformations. Moreover, both background and interictal nodular activity was closely similar in all recorded patients, suggesting that the morpho-functional organization of all nodules was similar, notwithstanding their variable extent and location; nodules also had uniform neuropathologic characteristics (see below) further supporting this conclusion.

**Surgery**

Seven of the patients operated on in this series became seizure-free after surgery and remained so at least a year later (Engel class Ia). All seven patients had unilateral heterotopia with similar clinical presentation, and the excellent surgical outcomes appear independent of whether the location was subependymal or subcortical. Less favourable outcomes were obtained in the three patients with bilateral (symmetrical or asymmetrical) nodular heterotopia. Published patients with nodular heterotopia operated on to relieve epilepsy generally had poor outcomes. In the study by Dubeau et al. (1995) only two of the seven patients achieved Engel class I, both of whom had unilateral nodules. Of the 10 patients published by Li et al. (1997), seven were bilateral and three unilateral, and bilaterality may be one reason for the poor overall results in this series; another reason might be that a standard temporal resection was performed in all cases, while in our series the surgery was based on the SEEG findings. We agree with Li et al. (1997) that clinical and electrographic features pointing to a temporal lobe origin of seizures are often misleading in nodular heterotopia. It is also evident from published experience that temporal resection does not usually result in long-term cessation of seizures in these conditions. However, we do not agree that nodular heterotopia should be considered per se a contraindication for surgery, as suggested by these authors. Our data indicate that good outcomes can be achieved for unilateral...
heterotopia when the surgery is guided by a careful invasive presurgical study. Our experience suggests that the overlying cortex is usually part of the epileptogenic zone and should be ablated. In some cases, part of the nodular formation was left in situ, yet outcomes were excellent. We stress, however, that our experience with bilateral heterotopia was less positive, although reductions in seizure frequency were obtained in all three bilateral cases.

**Neuropathology**

As noted, neuropathological studies on tissue from epileptic patients with nodular heterotopia are rare. Santi and Golden (2001) reported on three female and two male fetuses with bilateral periventricular nodular heterotopia associated with other abnormalities, although the cases did not show the extensive periventricular heterotopia usually present in females with FLN1 mutations. The nodules had similar structures in all cases, while aggregates of macrophages were present around the nodules in four cases, and disorganized radial glia surrounded nodules in all cases. These findings suggest disruption of radial glial organization as a cause of the failure of cells to migrate from the ventricular zone. The first autopsy study of periventricular nodular heterotopia with a genetically proven FLN1 mutation was published recently by Kakita et al. (2002). Routine histological, extensive immunohistochemical, and tracing studies with a carbocyanine dye (DiI) were performed. The authors described a general organization of the heterotopic nodules similar to that reported here and also the study of Hannan et al. (1999) on hemispherectomy specimens from patients with nodular heterotopia and other brain malformations. In all of these cases the nodules consist of masses of neurons, without laminar organization, with well-defined boundaries, surrounded by white matter fibres, some of which infiltrate the nodules. Within the nodules, aggregates of small pyramidal neurons intermingle with neurons positive for calcium-binding proteins, suggesting the presence of different subpopulations of GABAergic interneurons. Thus, the final structure of the nodules seems to be independent of their aetiology and unrelated to whether the heterotopia is unilateral or bilateral, subependymal or subcortical.

In Kakita et al.’s autopsy case <1% of neurons were labelled by the calcium-binding proteins CB, CR and PV (Kakita et al., 2002). In the present series, and in the patients reported by Hannan et al. (1999), considerably greater proportions of interneurons were positive for these proteins. Hannan et al. (1999) found features suggesting interneuronal immaturity in the nodules, probably in relation to the fact that the patients were children, in whom neuronal maturation can be expected to have been delayed. We found that interneurons immunolabelled with calcium binding protein were not reduced, as also reported by Hannan et al. (1999).

The nodular interneurons in all but one case of our series were normal and closely resembled those in the overlying cortex, although the pyramidal neurons were morphologically immature in all cases, as well as sporting haphazardly oriented dendrites.

The epileptiform activity that characterizes nodules suggests the presence of intrinsically hyperexcitable circuitry due to an unbalanced excitatory–inhibitory system.

In a rat model of induced heterotopia, Chen and Roper (2003) found that the frequency of spontaneous and miniature inhibitory postsynaptic currents was significantly reduced in intranodular pyramidal neurons compared with control pyramidal neocortical neurons, hence suggesting impairment of inhibitory GABAergic circuitry. The autopsic study of Kakita et al. (2002) found a reduction of GABAergic interneurons within the periventricular nodules, again consistent with the hypothesis of impaired inhibitory GABAergic circuitry. In contrast, Hannan et al. (1999) suggested that GABAergic activity within the nodules could be excitatory rather than inhibitory, as occurs in neuronal circuitry during development (Ben Ari et al., 1997). Moreover, recent studies on the cortex of mature rats have demonstrated that activation of the GABAergic interneuronal network can be excitatory in certain circumstances (Gulledge and Stuart, 2003). Thus it is likely that within the nodules the GABAergic circuitry could be altered.

It is possible, however, that other mechanisms may be involved in hyperexcitability: a recent study on human nodular formations removed during epilepsy surgery found lower than normal expression of alpha-CaMKII kinase and, in cases with more extensive heterotopia, lower than normal expression of the NR2A/B regulatory subunit of the NMDA receptor (Battaglia et al., 2002), suggesting involvement of the excitatory system mediated by NMDA receptors. However, an NMDA-mediated mechanism does not exclude a GABA-mediated one; both could coexist, enhancing the excitatory–inhibitory imbalance within nodules.

Nodular heterotopia is currently considered to be the result of defective neuronal migration in early development. Dobyns et al. (1996) suggested that the gene product defective in BPNH (an actin-binding protein necessary for the movement of certain cells) is required to signal the end of neuroblast division, and that lack of this product results in the neuroblast over-proliferation that characterizes BPNH. A similar mechanism could be occurring in nodular heterotopia not arising from a defect in the FLN1 gene.

With regard to the structural organization of the cortex overlying nodular heterotopia, once again few data have been published. MRI studies frequently report polymicrogyria associated with nodular heterotopia. In seven patients of the present series, MRI showed abnormal gyration, with normal signal intensity; however, histological abnormalities consistent with typical polymicrogyria were not found. For these cases we prefer the terms polygyria or microgyria, to distinguish them from the histologically defined polymicrogyria.

In our previous study on three surgically resected epileptic patients, the two with nodular heterotopia had a generally
normal neocortex, although there was cortical disruption in one at the point where a nodule impinged on the cortex (Sprefacio et al., 1992). In the autopsy case by Kakita et al. (2002) cortical dysplasia was present. In the present series, nine of the 10 patients presented various degrees of cortical dysplasia. Cortical dysplasia is also associated with reduced expression of calcium-binding proteins, particularly PV (De Felipe et al., 1993; Sprefacio et al., 2000). Whatever the aetiological mechanisms of nodular heterotopia, they seem frequently to involve the cortex, although the severity of cortical involvement is variable, and hence do not constitute a dual pathology. The altered cortex may originate seizures independently of, in synchrony with, epileptiform activity generated by nodules, as shown by the SEEG studies in our patients. Consistent with this observation DiI tracing has shown that fibres connect nodules to other nodules and to the cortex, in both genetically and non-genetically determined nodular heterotopia (Hannan et al., 1999; Kakita et al., 2002). A dominant role of the cortex in originating seizure activity in heterotopia is also supported by studies on the Tish rat (Chen et al., 2000).

Hippocampal sclerosis often accompanies nodular heterotopia. Raymond et al. (1995) reported hippocampal sclerosis in all five operated patients, while Dubeau et al. (1995) and Li et al. (1997) found hippocampal sclerosis in six of 10, and two of seven patients, respectively, operated on in the temporal lobe. In the present study none of the eight patients from whom hippocampal material was removed had hippocampal sclerosis, although gliosis was present in five. The aetiology of hippocampal sclerosis is debated. It may be the outcome of disrupted cortical development when the temporal lobe is the most affected. If such were the case the commonly used term dual pathology would be misleading.

Acknowledgements
We thank Marina De Negri for preparing the manuscript and Don Ward for help with the English. The research was supported by FIRB grant No. RBNE01NR34-008 from the Italian Ministry of Health, and by a grant from the Fondazione Banca del Monte di Lombardia.

References