Inhibitory neurons in the human epileptogenic temporal neocortex
An immunocytochemical study

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Summary
Immunocytochemical methods were used to study alterations in inhibitory neuronal circuits in human neocortex resected during surgical treatment of intractable temporal epilepsy associated or not with brain tumours. The epileptogenic cortex was characterized and divided into spiking or non-spiking zones by intraoperative electrocorticography (ECOG). The resected cortex was cut into blocks, sectioned and stained immunocytochemically for visualization of glutamic acid decarboxylase (GAD), the calcium-binding protein, parvalbumin (PV) and glial fibrillary acidic protein (GFAP). A variety of alterations in cortical neuronal circuits as revealed by immunocytochemical and histological methods were found. Similar alterations in inhibitory neuronal circuits appear to occur independently of the primary epileptogenic site and pathology associated with epilepsy, which suggests that there is possibly a common basic underlying mechanism that leads to seizure activity. These changes were apparently unrelated to ECOG findings at surgery, which bring into question the value of the use of interictal epileptic discharges recorded by ECOG to guide cortical resections. The most conspicuous and common change was the loss of chandelier cells. The finding that these cells are among the most vulnerable types of GABAergic interneurons in the epileptogenic temporal cortex indicates that they might be of great functional importance, since the axon terminals of chandelier cells are likely to exert powerful regulation of impulse generation in cortical pyramidal cells. Therefore, these cells might represent a key component in the aetiology of human epilepsy.

Keywords: focal epilepsy; brain tumours; glutamic acid decarboxylase; parvalbumin; gliosis

Abbreviations: ECOG = electrocorticography; GAD = glutamic acid decarboxylase; GFAP = glial fibrillary acidic protein; GluR2/3 = glutamate receptor subunits 2 and 3; GluR5/6/7 = glutamate receptor subunits 5, 6 and 7; PV = parvalbumin; WHO = World Health Organization

Introduction
Temporal lobe epilepsy is one of the most frequent types of human focal epilepsy. There is a variety of epileptogenic lesions and the most important hypotheses regarding the basic mechanisms of epilepsy in both humans and experimental animals are based on alterations (anatomical and/or chemical) of glutamatergic (excitatory) and GABAergic (inhibitory) cortical neuronal systems (Lloyd et al., 1986; Sherwin and van Gelder, 1986; van Gelder, 1987; Houser, 1991; Ribak, 1991; Avanzini et al., 1992; Hamberger and van Gelder, 1993). However, it is not yet known why cortical tissue becomes epileptogenic and why some patients can be well controlled on antiepileptic drugs, whereas others (the minority) are uncontrollable. In the latter group, a relatively high percentage can be surgically treated; the most common surgical procedure being the removal of the anterior temporal cortex, most of the amygdala and the anterior portion of the hippocampus (Olivier, 1992). The most frequent pathological change found in the resected tissue is so-called sclerosis of the hippocampus (e.g. Falconer, 1974; Meldrum and Bruton, 1992; Babb and Pretorius, 1993),
Fig. 1 (A) MRI of Patient H17 showing a pilocytic astrocytoma localized mesially in the right temporal lobe (arrow). (B) Lateral view of resected anterior temporal lobe from Patient H17, which includes the tumoural mass (arrow).

but the lateral temporal cortex is usually considered to be normal (e.g. Babb et al., 1984). However, it has been recently shown that in lateral human epileptogenic cortex with normal appearance in routine histopathological preparations there may be small regions or patches of decreased immunostaining for the calcium-binding protein PV and/or for the glutamate receptor subunits 2 and 3 (GluR2/3) and 5, 6 and 7 (GluR5/6/7) of the d. l.-α-amino-3-hydroxy-5-methyl-4-isoxazole proponic acid/kainate and kainate receptor subtypes (DeFelipe et al., 1993, 1994). Furthermore, using correlative light and electron microscopic immunocytochemical methods, these focal decreases have been shown to correspond to a fine disorganization in synaptic circuits which consists of an increase and decrease of presumptive excitatory and inhibitory synapses, respectively (DeFelipe et al., 1993; P. Marco and J. DeFelipe, unpublished work). In addition, PV immunocytochemistry labels a subpopulation of GABAergic interneurons which includes chandelier and basket cells. Because these two types of cells innervate the somata (and proximal dendrites) and axon initial segments of pyramidal cells, respectively, they are considered to be (in particular chandelier cells) the most powerful inhibitory interneurons in controlling pyramidal cell excitability (for review, see DeFelipe and Farías, 1992). Thus, PV immunoreactivity can be utilized as a useful tool to study abnormal synaptic circuits in the human epileptogenic cortex that may be particularly relevant to epileptogenesis (DeFelipe et al., 1993). Moreover, immunocytochemical studies of focal epilepsy using antibodies directed against the GABA-synthesizing enzyme, GAD in monkeys made experimentally epileptic, have shown a preferential loss of GABAergic neurons and axon terminals at epileptic foci (Ribak et al., 1979, 1982; Houser et al., 1986; Houser, 1991). It was hypothesized that this loss of inhibition could lead to epileptiform activity of cortical pyramidal neurons (Ribak et al., 1979).

In the present study, the major aims were (i) to investigate further possible alterations in the inhibitory neuronal circuitry that might occur in the human neocortex, using immunocytochemistry for PV and GAD on samples obtained during surgical treatment of temporal lobe epilepsy associated or not with brain tumours; and (ii) to compare these patterns of immunostaining with each other, and with intraoperative ECOG recordings (i.e. spiking or ‘active’ and non-spiking or ‘non-active regions’). In addition, these patterns of immunostaining and ECOG findings were also compared with cytoarchitectural features in thionin-stained sections adjacent to those used for immunocytochemistry, and with the patterns of immunostaining for GFAP. Immunocytochemistry for GFAP was used for detection of reactive astrocytes, which are a frequently detected by histopathological methods in epileptogenic cortex (e.g. Meldrum and Bruton, 1992).

Material and methods

Tissue preparation

Human cortical tissue was obtained, during surgical treatment, from 13 patients with intractable temporal lobe epilepsy associated (n = 4) or not associated (n = 9) with brain tumours. Informed consent was obtained in all cases. The line of excision was based on clinical evaluations, EEG studies (including foramen ovale electrodes recording and chronic monitoring from subdural grid electrodes in some cases), neuropsychological assessment, MRI signals, CT scans (in some cases), intraoperative ECOG, and the appearance of the tissue during surgery. MRI signals were normal in the group of patients without brain tumours. The probable primary epileptogenic site was localized (using the electrophysiological data) in the amygdalo-hippocampal region, where most interictal epileptiform activity and all the ictal activity were recorded, in all cases but one (Patient H10), in whom it was localized in the neocortex. However, there was a significant interictal activity arising from the
lateral temporal cortex in all cases. The four patients with brain tumours showed abnormal MRI signals either near the amygdala-hippocampal region (Patients H16 and H17) (Fig. 1) or in the lateral neocortex and white matter (Patients H9 and H11). The clinical data are summarized in Tables 1 and 2. Surgical procedures were performed under general anaesthetic. In the cases without brain tumours (except Patient H10), the resection was a tailored anterior temporal cortical resection including most of the amygdala and 1–3 cm of the hippocampus. In Patient H10 only a cortical resection was performed. In the tumour cases, the surgical procedure was directed both to mass lesion removal and to improve seizure control by cortical resection tailored to the extent of the epileptogenic zone determined by interictal epileptiform abnormalities on the intraoperative ECOG.

Tissue preparation has been described in detail in DeFelipe et al. (1993). Briefly, the neocortex was characterized and marked as spiking or non-spiking by extracellular recordings with grid electrodes at the time of surgery. All operative procedures and preparation of tissue samples were recorded with a video camera. The resected brain tissue was immediately immersed in a cold solution of 4% paraformaldehyde in phosphate buffer (0.1 M, pH 7.4), then photographed and cut into small blocks about 15×8×8 mm that included either spiking or non-spiking regions, as guided by the video camera recording. The blocks were then postfixed, sectioned and stained immunocytochemically for GAD, PV or GFAP as indicated below.

**Immunocytochemistry**

The blocks were transferred to a second fixative solution of 4% paraformaldehyde in phosphate buffer in which they remained for 24 h at 4°C. Thereafter, they were cut serially at 100 μm on a Vibratome. Sections were processed for immunocytochemistry for GAD, PV and GFAP. The sections were pretreated with a solution of ethanol and hydrogen peroxidase in phosphate buffer to remove endogenous peroxidase activity, washed in phosphate buffer, and then preincubated in 3% normal serum and 0.05% Triton X-100 in phosphate buffer for 3 h at room temperature. Then, the sections were transferred to the latter solution to which either anti-PV antibody (mouse monoclonal antibody from Swant, Bellinzona, Switzerland) (Celio et al., 1988), diluted 1:5000, anti-GAD antibody (rabbit polyclonal antibody, K-2 from Chemicon, Temecula, Calif., USA) (Kaufman et al., 1991), diluted 1:1000, or anti-GFAP antibody (rabbit polyclonal antibody from Sigma, St Louis, Miss., USA), diluted 1:50, were added. Sections were incubated for 24 h at 4°C. They were rinsed in phosphate buffer and transferred to a solution containing biotinylated horse anti-mouse or biotinylated goat anti-rabbit immunoglobulins (Vector Labs, Burlingame, Calif., USA) for 1 h at room temperature. The sections were washed several times in phosphate buffer and incubated at room temperature in avidin–biotin–peroxidase complex (Vector Labs) for 1 h. After another series of washes, they were transferred to a solution of 0.05% 3,3′-diaminobenzidine tetrahydrochloride and 0.01% hydrogen peroxide in phosphate buffer for 2–3 min. The sections were then washed and osmicated in 0.02% osmium tetroxide, washed again and finally dehydrated, cleared with xylene and coverslipped with DePex mounting medium (BDH, Poole, UK). Sections adjacent to those used for immunocytochemistry were stained with thionin. Some tissue samples from medial temporal structures and lateral neocortex were embedded in paraffin, sectioned and stained with haematoxylin and eosin for the neuropathological evaluation of the removed tissue. Control sections for immunocytochemistry were processed as above but with primary antiserum replaced with normal serum, or in the case of PV immunocytochemistry with primary antiserum adsorbed with an excess of PV (Swant). No significant staining was detected under these control conditions.

Control tissue sections consisted of post-mortem human neocortical tissue (area 38 of Brodmann) from two individuals (C1 and C2) with no known neurological disease. These were kindly supplied by Dr C. Bouras (Geneva, Switzerland). The characteristics of these human samples were C1, 70-year-old male (2 h post-mortem delay); C2, 91-year-old male (6 h post-mortem delay). These samples were fixed and processed as described for the biopsies.

**Results**

**Immunocytochemistry for GAD and PV in the normal neocortex fixed by immersion: controls**

The patterns of immunostaining for GAD and PV in sections from post-mortem human neocortical tissue (control tissue sections) which were processed identically and in parallel to those sections obtained at biopsy from epileptic patients, were consistently homogeneous; i.e. no patches of decreased immunostaining were observed. Also the pattern of labelling of neurons and axonal plexuses was similar to that previously described by other authors in the neocortex of a variety of species (see Discussion).

**Epileptogenic neocortex cases not associated with brain tumours**

**Histopathology of medial temporal structures**

While cutting brain tissue into small blocks before postfixation (see Materials and methods), it was noticed that the cortex and white matter of the parahippocampal gyrus in the sample from Patient H12 were indurated, whereas the texture of the other samples was normal. Neuropathological examination of the removed hippocampus and/or parahippocampal gyrus showed significant histopathological findings in Patients H7, H12, H25, H26 and H35 (see Table 1). In Patients H27 and H18 no significant pathological changes were found (although in the case of Patient H18 the hippocampus was not available for pathological assess-
### Table 1  
Summary of clinical and surgical data in cases not associated with brain tumours

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)/sex</th>
<th>Side</th>
<th>Age of onset (years)</th>
<th>Possible aetiology</th>
<th>Seizure frequency</th>
<th>Seizure type</th>
<th>Neurological examination</th>
<th>Anti-epileptic drugs</th>
<th>Neuropsychological examination (IQ/hemispheric dominance)</th>
<th>Date of surgery (month/year)</th>
<th>Length of weight (g) of removed brain tissue</th>
<th>Neuropathological findings</th>
<th>Surgical results</th>
</tr>
</thead>
<tbody>
<tr>
<td>H7</td>
<td>13/M</td>
<td>Right</td>
<td>2</td>
<td>Unknown</td>
<td>Weekly</td>
<td>Partial complex</td>
<td>Deaf and dumb, right-handed</td>
<td>CBZ</td>
<td>&lt;70/left</td>
<td>2/1992</td>
<td>5.0/24.3</td>
<td>Neuronal loss and gliosis in the hippocampus and parahippocampal gyrus</td>
<td>Seizure-free</td>
</tr>
<tr>
<td>H10</td>
<td>38/M</td>
<td>Left</td>
<td>15</td>
<td>Unknown</td>
<td>Weekly</td>
<td>Partial complex, secondary generalized tonic-clonic</td>
<td>Normal, right-handed</td>
<td>PHT, PRM</td>
<td>7/1992</td>
<td>2.8/7.0</td>
<td>Neuronal loss and gliosis in the hippocampus and parahippocampal gyrus</td>
<td>Exceptional seizures</td>
<td></td>
</tr>
<tr>
<td>H12</td>
<td>35/M</td>
<td>Right</td>
<td>19</td>
<td>Complicated delivery</td>
<td>Daily</td>
<td>Partial complex, secondary generalized tonic-clonic</td>
<td>Normal, right-handed</td>
<td>CBZ, VPA</td>
<td>9/1992</td>
<td>4.4/25.1</td>
<td>Neuronal loss and gliosis in the hippocampus and parahippocampal gyrus</td>
<td>Seizure-free</td>
<td></td>
</tr>
<tr>
<td>H18</td>
<td>31/F</td>
<td>Left</td>
<td>24</td>
<td>Complicated delivery</td>
<td>Daily</td>
<td>Partial complex, secondary generalized tonic-clonic</td>
<td>Normal, right-handed</td>
<td>CBZ, PB</td>
<td>1/1993</td>
<td>4.3/26.5</td>
<td>Seizure-free</td>
<td>Seizure-free</td>
<td></td>
</tr>
<tr>
<td>H25</td>
<td>44/M</td>
<td>Right</td>
<td>28</td>
<td>Unknown</td>
<td>Weekly</td>
<td>Partial complex</td>
<td>Normal, right-handed</td>
<td>PHT</td>
<td>6/1993</td>
<td>4.2/27.0</td>
<td>Seizure-free</td>
<td>Neuronal loss in the hippocampus; calcifications in the parahippocampal gyrus</td>
<td></td>
</tr>
<tr>
<td>H26</td>
<td>32/M</td>
<td>Right</td>
<td>2</td>
<td>?Viral encephalitis</td>
<td>Daily</td>
<td>Partial complex, secondary generalized tonic-clonic</td>
<td>Normal, right-handed</td>
<td>PB, PHT</td>
<td>6/1993</td>
<td>5.3/30.7</td>
<td>Neuronal loss in the parahippocampal gyrus (hippocampus not analysed)</td>
<td>Seizure-free</td>
<td></td>
</tr>
<tr>
<td>H27</td>
<td>29/M</td>
<td>Right</td>
<td>1</td>
<td>Complicated delivery: multiple febrile seizures</td>
<td>Daily</td>
<td>Partial complex, secondary generalized tonic-clonic</td>
<td>Normal, right-handed</td>
<td>CBZ, PB, VPA</td>
<td>6/1993</td>
<td>4.2/24.0</td>
<td>No significant alterations</td>
<td>Important seizure frequency decrease</td>
<td></td>
</tr>
<tr>
<td>H34</td>
<td>19/F</td>
<td>Left</td>
<td>11</td>
<td>Unknown</td>
<td>Weekly</td>
<td>Partial complex</td>
<td>Normal, right-handed</td>
<td>PHT, PRM</td>
<td>10/1994</td>
<td>3.3/11.4</td>
<td>No data available</td>
<td>Seizure-free</td>
<td></td>
</tr>
<tr>
<td>H35</td>
<td>30/F</td>
<td>Right</td>
<td>7</td>
<td>Unknown</td>
<td>Daily</td>
<td>Partial complex</td>
<td>Partial complex</td>
<td>Normal, right-handed</td>
<td>CBZ, VGB</td>
<td>11/1994</td>
<td>6.7/31.8</td>
<td>Gliosis in the parahippocampal gyrus</td>
<td>Seizure-free</td>
</tr>
</tbody>
</table>

Patient H10 was surgically treated only with a cortical resection. Therefore, the medial temporal structures were left intact. CBZ = carbamazepine; PB = phenobarbital; PHT = phenytoin; PRM = primidone; VGB = vigabatrin; VPA = valproate. *From the temporal pole; ¹In the hippocampus and parahippocampal gyrus.
Table 2 Summary of clinical and surgical data in cases associated with brain tumours

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)/sex</th>
<th>Side</th>
<th>Age of onset (years)</th>
<th>Possible aetiology</th>
<th>Seizure frequency</th>
<th>Seizure type</th>
<th>Neurological examination</th>
<th>Anti-epileptic drugs</th>
<th>Neuropsychological examination IQ/hemispheric dominance</th>
<th>Date of surgery (month/year)</th>
<th>Length (cm)/weight (g) of removed brain tissue</th>
<th>Neuropathological findings</th>
<th>Surgical results</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9</td>
<td>19/M</td>
<td>Left</td>
<td>5</td>
<td>Tumour</td>
<td>Daily</td>
<td>Partial complex, left-handed</td>
<td>Normal, right-handed</td>
<td>PRM</td>
<td>89/right</td>
<td>6/1992</td>
<td>5.4/18.4</td>
<td>Ependymoma</td>
<td>Seizure-free</td>
</tr>
<tr>
<td>H11</td>
<td>30/F</td>
<td>Right</td>
<td>7</td>
<td>Tumour</td>
<td>Daily</td>
<td>Partial complex, right-handed</td>
<td>Normal, right-handed</td>
<td>PHT</td>
<td>70/left</td>
<td>7/1992</td>
<td>5.0/20.5</td>
<td>Anaplastic astrocytoma (WHO grade III)</td>
<td>Unchanged seizures</td>
</tr>
<tr>
<td>H16</td>
<td>23/F</td>
<td>Right</td>
<td>14</td>
<td>Tumour</td>
<td>Daily</td>
<td>Partial complex, right-handed</td>
<td>Normal, right-handed</td>
<td>CBZ, PB, PHT, CLB</td>
<td>110/left</td>
<td>12/1992</td>
<td>4.5/32.0</td>
<td>Pilocytic astrocytoma (WHO grade I)</td>
<td>Exceptional seizures</td>
</tr>
<tr>
<td>H17</td>
<td>18/M</td>
<td>Right</td>
<td>17.5</td>
<td>Tumour</td>
<td>Monthly</td>
<td>Partial complex, secondary generalized tonic-clonic</td>
<td>Normal, right-handed</td>
<td>PHT</td>
<td>125/left</td>
<td>12/1992</td>
<td>5.5/22.8</td>
<td>Pilocytic astrocytoma (WHO grade I)</td>
<td>Seizure-free</td>
</tr>
</tbody>
</table>

CBZ = carbamazepine; CLB = clobazam; PB = phenobarbital; PHT = phenytoin; PRM = primidone. *From temporal pole; †type of tumour.
ment). No data were available for Patient H34. These changes consisted mainly of neuronal cell loss and/or gliosis (Fig. 2A and B) in the hippocampus and/or parahippocampal gyrus. In Patient H25, the pathology found was neuronal cell loss and gliosis in the hippocampus and calcifications in the parahippocampal gyrus (Fig. 3). The amygdala were only available for pathological examination in Patients H7 and H25 and, in both cases, neuronal cell loss and gliosis were found.

Cytoarchitecture and patterns of immunostaining for PV, GAD and GFAP in the lateral neocortex

Tissue samples used for immunocytochemistry were from anterior portions of the temporal lobe (areas 38, 20 and 21 of Brodmann). In thionin-stained sections from the 36 cortical blocks from the nine patients examined (Table 3), 25 blocks presented a normal cytoarchitecture, eight displayed gliosis and three showed small regions with cell loss (focal cell loss) (Fig. 4). Within the regions showing cell loss, many of the remaining neurons were darkly stained and shrunken (Fig. 4D) compared with adjacent normal cortex (Fig. 4C) and some of them showed pericellular incrustations (Fig. 5).

Sections from 32 blocks were processed for GFAP immunocytochemistry. Numerous GFAP-positive cells were found in sections from only seven blocks in at least one cortical layer as compared with the other blocks which showed few GFAP-positive cells in the grey matter. Therefore, we distinguished two patterns of GFAP immunostaining in the grey matter: pattern 0 (normal, see Discussion) when few or no GFAP-positive cells were present, and pattern + + (abnormal) when numerous cells were stained. It was notable that only a few blocks showed gliosis in Nissl stained sections and also only a few blocks showed pattern + + of GFAP, and that different cortical layers were affected (layer VI and layers I–III, respectively) (see Table 3).

We distinguished four main patterns of PV-immunostaining (patterns A, B, C and D) (Fig. 6), as have previously been described in human epileptogenic neocortex (DeFelipe et al., 1993). Pattern A was characterized mainly by the labelling of numerous nonspiny interneurons (including chandelier cells and basket cells) and processes and by the labelling of a dense immunoreactive band in the middle layers (Fig. 6A). This band is made up of immunoreactive cell somata, processes and puncta [originating, in part, from the axon terminals of nonspiny interneurons and, in part, from terminals of thalamocortical neurons (see DeFelipe et al., 1993; del Río and DeFelipe, 1994)]. The three other main patterns (Fig. 6B–D) of immunostaining showed decreased immunoreactivity to a variable extent and were considered abnormal. In the present study, the analysis of a larger number of cortical blocks from more patients has allowed us to make a more detailed definition compared with our previous studies of these altered patterns, particularly pattern C. Patterns B (Fig. 6B) and D (Fig. 6D) were characterized by a reduction in immunoreactivity (in intensity of staining and number of immunoreactive elements) only in the layers above the superficial half of layer III or in all layers (from layer I to layer VI), respectively. These two patterns and, in particular, pattern B, usually affected...
Neocortical inhibitory cells in epilepsy

Fig. 3 (A) Low power photomicrograph of a section through the parahippocampal gyrus stained with haematoxylin and eosin from Patient H25, showing calcifications (arrows) in the deeper layers of the parahippocampal gyrus. (B and C) Higher magnification of A. These calcium deposits (arrows) display a concentric rings structure. Scale bar = 236 μm for A, 10 μm for B and C.

relatively large segments of cortex (often >1000 μm wide). Pattern C was the most outstanding because this pattern of decreased immunoreactivity appeared in rather small regions (often 200–1000 μm wide), while the surrounding cortex showed a normal pattern of immunostaining (Fig. 6C). The decreased immunoreactivity consisted of a virtual absence of immunoreactive neurons (whereas in patterns B and D there are relatively numerous immunoreactive neurons, although lightly stained) and a reduction in immunoreactive processes, affecting mainly the chandelier cell axon terminals. Furthermore, the decreased immunostaining can affect a single or several of the superficial, middle or deep cortical layers. (In the present study we referred to these patches of decreased immunostaining as pattern C with a subscript indicating the layers showing that decrease.) Finally, block 1a from Patient H18 showed a pattern of immunostaining that, at first glance, was indistinguishable from pattern A, but a closer inspection showed a virtual lack of chandelier cell axon terminals in layers II and III. This kind of pattern was commonly found in cases associated with brain tumours (see below). We referred to this pattern as A (–Ch) with a subscript indicating the layers with such reduction. In general, in patterns B and D all types of PV-positive cells appear to be equally affected, whereas in pattern C the consistent lack of chandelier cell terminals is notable while the number of terminal-like puncta may vary from a moderate or severe reduction to normal. Therefore, chandelier cells are among the most vulnerable. As shown in Table 3, of the 36 blocks processed for PV immunocytochemistry, 29 showed a single pattern (27 pattern A; two pattern B), whereas the other seven blocks displayed a mixture of patterns [five patterns A and C; one pattern A(–Ch) and C; one pattern B and D]. Furthermore, pattern A is the predominant pattern in the blocks showing patterns A and C. Thus, the normal pattern of PV immunostaining is the most frequently found.

In sections stained immunocytochemically for GAD, two
Table 3

Summary of immunocytochemical results and cytoarchitecture (in adjacent serial sections unless otherwise specified) in non-spiking and spiking cortical regions from patients without brain tumours

<table>
<thead>
<tr>
<th>Sample location within ECOG*</th>
<th>Patient</th>
<th>Pattern staining in adjacent serial sections to those immunocytochemically stained for GAD and PV, except when indicated by (ns), that means that the sections were non-adjacent.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block 2: area 38</td>
<td>H25</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 5: area 21</td>
<td>H27</td>
<td>Spiking</td>
</tr>
<tr>
<td>Block 6: area 21</td>
<td>H35</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 7: area 21</td>
<td>H26</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 10: area 21</td>
<td>H18</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 4: area 21</td>
<td>H7</td>
<td>Spiking</td>
</tr>
<tr>
<td>Block 11: area 38</td>
<td>H10</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 2: area 38</td>
<td>H12</td>
<td>Spiking</td>
</tr>
<tr>
<td>Block 5: area 38</td>
<td>H12</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 2: area 20</td>
<td>H12</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 4: area 20</td>
<td>H12</td>
<td>Non-spiking</td>
</tr>
<tr>
<td>Block 8: area 20</td>
<td>H12</td>
<td>Non-spiking</td>
</tr>
</tbody>
</table>

Pattern staining

- GAD
- PV

Cytoarchitecture

- Normal
- Normal (ns)
- Abnormal
- Abnormal (ns)
- Gliosis
- Gliosis (ns)
- Focal cell loss
- Focal cell loss (ns)

Legend:

- Normal
- Normal (ns)
- Abnormal
- Abnormal (ns)
- Gliosis
- Gliosis (ns)
- Focal cell loss
- Focal cell loss (ns)
Fig. 4 Photomicrographs of a 100 μm thick vibratome section through area 38 stained with thionin from Patient H34 (block 1), showing a small region with severe neuronal cell loss: (A and B) low (A) and higher magnification (B) photomicrographs illustrating a region with focal neuronal cell loss (asterisks) in layer III; (C and D) photomicrographs illustrating normal looking neurons (C) from a normal region adjacent to the area of neuronal cell loss (D) which shows fewer and abnormal looking neurons (arrows) compared with C. Scale bar = 509 μm for A, 280 μm for B, 50 μm for C and D.

main patterns of immunostaining (a and c) were distinguished. Pattern a (Fig. 7) was characterized by the presence of a relatively large number of immunostained cell somata (particularly in layers II–IV), and numerous terminal-like puncta throughout all layers. The unstained somata of pyramidal cells were frequently outlined by numerous
immunoreactive puncta (Fig. 7C and D). This pattern was similar to that previously described in the normal primate neocortex (e.g. Houser et al., 1984) and was considered to be normal. Pattern c was considered to be abnormal (see Discussion) and was characterized by the decrease in both immunoreactive puncta and, particularly, of cell somata (Fig. 8). As occurred with pattern C of the PV immunostaining, the decrease in GAD immunostaining was found to affect a single layer or several of the superficial, middle or deep cortical layers and appeared in small regions of the same size as pattern C of the PV immunostaining. Therefore, we used the same terminology indicating the layers showing that decrease by subscripts. It was notable that only few patterns c were found (Table 3): only five out of 32 blocks processed for GAD immunocytochemistry showed pattern c.

Correlation between patterns of immunostaining for PV and GAD
As shown in Table 3, in all cases where the PV staining pattern was normal (pattern A) in a given section, a normal pattern for GAD immunocytochemistry (pattern a) was found in the adjacent section, and that when pattern C of PV immunoreactivity was present in a section, this decrease was mirrored by coincident decrease of GAD immunoreactivity (pattern c) in the adjacent section. Therefore, there was a complete coincidence of patterns A and a, and C and c for PV and GAD immunoreactivities in the same regions. However, this correlation did not occur with patterns B and D of PV immunostaining, since in the corresponding adjacent sections that were stained for GAD the pattern of immunostaining was normal (pattern a).

Correlation between patterns of immunostaining for GAD and PV with the pattern of immunostaining for GFAP and cytoarchitecture
Comparison of sections immunocytochemically stained for PV or for GAD with sections that had been stained with thionin or immunocytochemically for GFAP, showed that a given pattern of immunostaining for PV or GAD was apparently not associated with any particular cytoarchitectural characteristic nor with a high or small number of GFAP-positive cells, except the correlation between patterns C and c and focal cell loss (Table 3). Examination of adjacent serial sections that had been stained with thionin, revealed that the regions showing these abnormal patterns were always associated with cortical tissue showing focal cell loss, with surrounding tissue being of normal appearance. Recently, this correlation with patches of decreased immunostaining has been demonstrated more directly after examination of

Fig. 5 High power photomicrographs of neurons within the region showing neuronal cell loss illustrated in Fig. 4D. The neurons are darkly stained and shrunken and show pericellular incrustations (arrows). Note the staining of proximal dendritic processes (open arrows) in B. Scale bar = 10 μm for A and B.
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Fig. 6 Photomicrographs showing the main patterns of PV immunostaining observed in the human epileptogenic cortex. Pattern A immunostaining is considered to be normal, whereas patterns B–D, showing decreased immunostaining are considered to be abnormal. Note, in C decreased immunoreactive elements in a small region in layer IIIA (asterisk). Scale bar = 200 μm for A–D.

toluidine blue stained semi-thin (2 μm thick) plastic sections obtained after resectioning of plastic embedded sections (P. Marco and J. DeFelipe, unpublished observations). In thionin-stained sections, this focal cell loss was seen as a moderate or severe decrease in neurons (Fig. 4), and the decrease affected (at least in the severe cases) all kinds of neurons (i.e. pyramidal cells and nonpyramidal cells). Often the regions with focal cell loss were so small that they were very difficult to identify in thionin-stained sections. However, once a region with a pattern c of GAD or pattern C of PV immunostaining was identified (which is a change that is easily detected), then it was readily identified as a decrease in cells in the adjacent thionin-stained section (Fig. 4).

Correlation of intraoperative ECOG with cytoarchitecture and patterns of immunostaining for GAD, PV and GFAP

As shown in Table 3, both non-spiking and spiking regions (as determined by intraoperative ECOG) displayed either normal or abnormal cytoarchitectural characteristics or patterns of immunostaining for PV, GAD or GFAP. Therefore, there was a lack of correlation between electrocortico-graphical and anatomical findings.

Epileptogenic neocortex cases associated with brain tumours

Histopathology

In these patients (Patients H9, H11, H16 and H17), MRI signals revealed the existence of intracerebral masses, which after surgical removal and neuropathological examination, were confirmed to be tumours (Fig. 1). Tumours were classified according to Russell and Rubinstein (1989) and the World Health Organization (WHO) (Kleihues et al., 1993). In Patients H16 and H17, the tumours were located near the amygdalo-hippocampal region, whereas in Patients H9 and H11 the tumours affected the lateral neocortex and white matter. The tumours were identified (Table 2) as pilocytic astrocytomas (WHO grade I) (Patients H16 and H17), ependymoma (Patient H9) and anaplastic astrocytoma (WHO grade III) (Patient H11). The long duration of the symptoms (23 years) in the latter case could be explained by a malignant transformation of a previous low grade tumour into a higher grade (e.g. see Wolf et al., 1993).
Fig. 7 GAD immunostaining through layers I–VI of area 21 from Patient H18 (block 7), showing pattern a (normal) of GAD immunostaining. (A) Low power photomicrograph showing numerous immunostained cell somata throughout all layers (B) Higher magnification photomicrograph through layers IIIA–IV, illustrating the variety of morphological types of GAD-positive neurons (arrows). (C) and (D) Photomicrograph through layer IIIB, showing numerous immunoreactive puncta. Note the unstained somata of pyramidal cells (p) outlined by immunoreactive puncta. Scale bar = 258 μm for A, 100 μm for B, 32 μm for C and D.
Neocortical inhibitory cells in epilepsy

I

Fig. 8 (A) GAD immunostaining through layers IIIA–V of area 21 from Patient H18 (block 5), showing pattern c (abnormal) of GAD immunostaining. Arrow indicates a focal decrease of immunoreactivity. (B and C) Higher magnification photomicrographs of the centre (B) and periphery (C) of the region with decreased immunostaining. Note the decrease of both immunoreactive puncta and, particularly, of cell somata, in the central part. Scale bar = 311 μm for A, 54 μm for B, 50 μm for C.

Cytoarchitecture and patterns of immunostaining for PV, GAD and GFAP in the lateral neocortex

Tissue samples used for immunocytochemistry were non-tumoural samples (confirmed by neuropathological assessment) from anterior portions of the temporal lobe (areas 38 and 21 of Brodmann) which were located at a distance from the tumour. In thionin-stained sections from the nine cortical blocks from the four patients examined (Table 4) it was found that four blocks displayed a normal cytoarchitecture (Fig. 9A), two showed gliosis and three showed a small region with focal cell loss. In sections processed for GFAP immunocytochemistry from the seven blocks examined, numerous GFAP-positive cells and processes were found in all but two blocks.

In sections immunocytochemically stained for GAD and PV, the same patterns of immunostaining as in cases not
Table 4: Summary of immunocytochemical results and cytoarchitecture (in adjacent serial sections) in non-spiking and spiking cortical regions from patients with brain tumours

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample location within the neocortex</th>
<th>ECOG</th>
<th>Pattern staining GAD*</th>
<th>Sample location within the neocortex</th>
<th>Pattern staining PV*</th>
<th>Cytoarchitecture*</th>
<th>GFAP staining*</th>
</tr>
</thead>
<tbody>
<tr>
<td>H9</td>
<td>Block 3: area 38</td>
<td>Spiking</td>
<td>–</td>
<td>Block 3: area 38</td>
<td>A&lt;sup&gt;-Ch&lt;/sup&gt;&lt;sub&gt;H-V&lt;/sub&gt;</td>
<td>Normal</td>
<td>–</td>
</tr>
<tr>
<td>H11</td>
<td>Block 2: area 38</td>
<td>Non-spiking</td>
<td>a&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Block 2: area 38</td>
<td>A&lt;sup&gt;-Ch&lt;/sup&gt;&lt;sub&gt;H-V&lt;/sub&gt;</td>
<td>Gliosis&lt;sub&gt;H-V&lt;/sub&gt;</td>
<td>+&lt;sub&gt;H-V&lt;/sub&gt;</td>
</tr>
<tr>
<td>H16</td>
<td>Block 1a: area 38</td>
<td>Non-spiking</td>
<td>–</td>
<td>Block 1a: area 38</td>
<td>A&lt;sup&gt;-Ch&lt;/sup&gt;&lt;sub&gt;H-III&lt;/sub&gt;</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Block 3: area 38</td>
<td>Non-spiking</td>
<td>–</td>
<td>Block 3: area 38</td>
<td>A&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Normal†</td>
<td>+&lt;sub&gt;H-V&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Block 4a: area 38</td>
<td>Non-spiking</td>
<td>a&lt;sup&gt;+&lt;/sup&gt;,&lt;sub&gt;H-V&lt;/sub&gt;</td>
<td>Block 4a: area 38</td>
<td>A&lt;sup&gt;-&lt;/sup&gt;,&lt;sub&gt;H-V&lt;/sub&gt;</td>
<td>Normal†</td>
<td>+&lt;sub&gt;H-V&lt;/sub&gt;</td>
</tr>
<tr>
<td>H17</td>
<td>Block 1: area 21</td>
<td>Non-spiking</td>
<td>–</td>
<td>Block 1: area 21</td>
<td>A&lt;sup&gt;+&lt;/sup&gt;,&lt;sub&gt;B&lt;/sub&gt;</td>
<td>Normal</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Block 2: area 21</td>
<td>Non-spiking</td>
<td>a&lt;sup&gt;+&lt;/sup&gt;</td>
<td>Block 2: area 21</td>
<td>A&lt;sup&gt;-&lt;/sup&gt;</td>
<td>Normal†</td>
<td>+&lt;sub&gt;H-V&lt;/sub&gt;</td>
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<tr>
<td></td>
<td>Block 7: area 21</td>
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<td>a&lt;sup&gt;+&lt;/sup&gt;,&lt;sub&gt;L-V&lt;/sub&gt;</td>
<td>Block 7: area 21</td>
<td>A&lt;sup&gt;-&lt;/sup&gt;,&lt;sub&gt;B, L-V&lt;/sub&gt;</td>
<td>Normal†</td>
<td>0</td>
</tr>
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</table>

For abbreviations and symbols, see Table 3.
Neocortical inhibitory cells in epilepsy

Fig. 9 Parvalbumin immunostaining through area 38 of the human epileptogenic neocortex in cases associated with brain tumours. (A and B) Photomicrographs of paired surfaces of adjacent sections, one stained with thionin (A) and the other for PV (B), from block 1a of Patient H16. (C) Photomicrograph of a section stained for PV from block 2 of Patient H11. Asterisks indicate the dense immunoreactive band extending from the lower half of layer III to layer IV typical of the normal temporal cortex. Arrows indicate immunoreactive neurons in layers II and V, which are also shown in Fig. 10 at a higher magnification. Scale bar = 300 μm for A, B and C.

Correlation between patterns of immunostaining for PV and GAD

As occurred in the cases not associated with tumours, there was a complete coincidence of patterns A and \( a \), and C and \( c \) for PV and GAD immunoreactivities, respectively in the same regions (Table 4). For comparison between the other abnormal patterns of immunostaining for PV and GAD, unfortunately sections from only three blocks showing abnormal patterns of PV immunostaining (two showing a pattern A (–Ch) and the other a pattern B) were available for GAD immunocytochemistry. In these sections, the pattern of immunostaining for GAD was normal (pattern \( a \)), as in the cases not associated with tumours.

Correlation between patterns of immunostaining for GAD and PV with the pattern of immunostaining for GFAP and cytoarchitecture

Comparison of sections immunocytochemically stained for PV or for GAD with sections that had been stained with thionin or immunocytochemically for GFAP showed a lack of correlation between normal and altered patterns, except the correlation between patterns C and \( c \) and focal cell loss (Table 4). Again, these results were the same as in the cases not associated with tumours. The only difference found between the two groups of patients was that in all cortical blocks from patients with brain tumours at least one of
Fig. 10 Higher magnification photomicrographs of Figs 9B (A and C) and 9C (B and D), showing patterns A(-Ch)_{II-III} and A(-Ch)_{IV-VI} of PV immunostaining, respectively (see text for further details). Open arrows in A and B indicate the same immunoreactive neurons in layer II as in 9B and C. Note the lack of immunoreactive chandelier cell terminals (short, vertically oriented rows of puncta). Open arrows in C and D indicate the same immunoreactive neurons in layer V as in Fig. 9B and C. Note that in C there is a large number of immunoreactive chandelier cell terminals (arrows), whereas in the same layer in D there is a dramatic decrease of these terminals. Scale bar = 100 μm for A and B, 204 μm for C and D.
the patterns of immunostaining or the cytoarchitecture was abnormal.

In summary, the abnormalities found in the epileptogenic neocortex of patients with brain tumours were the same as in the cases without brain tumours, but in the former group of patients the alterations were more commonly found than in the other group.

**Correlation of ECOG with cytoarchitecture and patterns of immunostaining for GAD, PV and GFAP**

All cortical regions examined except one displayed a non-spiking (normal) activity as determined by intraoperative ECOG. However, all blocks showed an abnormal cytoarchitecture or altered patterns of immunostaining. Therefore, the lack of correlation between abnormal circuitry and abnormal ECOG in these cases was even more clear than in the non-tumoural cases.

**Discussion**

In the present study we have made three main observations. First, in human neocortex resected during surgical treatment of intractable temporal lobe epilepsy associated or not with brain tumours, multiple small regions with abnormal patterns of immunostaining for PV and GAD were commonly found. Secondly, there is a variety of changes in inhibitory circuits and these changes may, in certain regions, affect the whole population of inhibitory neurons, whereas in other regions they may affect a subpopulation of these neurons (in particular chandelier cells) more selectively. Thirdly, there were no consistent changes in any of the parameters examined that could be correlated with the normal or abnormal ECOG characteristics of the samples at surgery. These abnormal patterns of immunostaining were unlikely to be due to uneven fixation in different portions of the tissue block and/or differences in the penetration of immunocytochemical reagents, first because control sections from post-mortem human neocortical tissue show no such patches of decreased immunostaining, and secondly because previous correlative light and electron microscopic studies of regions showing focal decreases in PV-immunoreactivity have shown that, in these regions, there is a disorganization in synaptic circuitry (see below).

**Immunocytochemical and cytoarchitectural changes: what is normal and abnormal?**

The main limitation in these kinds of studies is, of course, that we do not have data about patterns of immunostaining for GAD and PV from biopsy samples of the strictly normal human neocortex. It is generally thought that cortical regions that had been resected in order to gain access to tumours or other abnormalities located deep in the brain are good controls. However, possible damage to subcortical projection pathways to the neocortex, as well as other alterations (e.g. drug therapy) which may affect the neocortex, should not be disregarded. Furthermore, tumours may lead to changes that eventually cause epilepsy after variable delays (Ketz, 1974; Spencer et al., 1984; Morris and Estes, 1993). Another more common source of neurochemical and anatomical data on non-pathological human neocortex comes from autopsy samples. It is true that a relatively long post-mortem delay may severely affect levels of amino acid such as GABA (e.g. Lloyd et al., 1986), but does not apparently affect immunostaining for certain antigens (e.g. PV immunostaining; see Blümcke et al., 1990; Ferrer et al., 1991; Hof et al., 1991). Furthermore, there are numerous studies on the cerebral cortex in a variety of other primates and non-primates mammals, using the same or different antibodies as in the present study (e.g. Celio, 1986; Ribak, 1978; Houser et al., 1984; DeFelipe et al., 1989; Esclapez et al., 1994). In our control material (autopsy samples processed as the biopsies) and other studies on normal neocortex, there is no indication of patches of decreased immunostaining for GAD and PV as found in the epileptic neocortex. Thus, patterns A and a of immunostaining for PV and GAD are considered to be normal because these patterns are virtually identical to the patterns of immunostaining found in the normal neocortex of a variety of species, whereas the patterns showing decreased of immunostaining (pattern c of GAD immunostaining and patterns B, C and D of PV immunostaining) are considered to be abnormal (DeFelipe et al., 1993; see below). In the case of immunocytochemistry for GFAP, the staining of grey matter fibrous astrocytes (which are immunoreactive for GFAP) varies widely among individuals (e.g. Hansen et al., 1987), but this number is rather small in the normal brain of relatively young individuals (Meldrum and Bruton, 1992) as for the cases in the present study. Therefore, we considered the appearance of many GFAP-positive cells (pattern + +) to be an abnormal pattern. Finally, since mild neuronal cell loss or gliosis is difficult to evaluate due to the great variation in the numbers of cortical glial cells and neurons between individuals (e.g. Henderson et al., 1980), the cytoarchitecture in thionin-stained sections was considered abnormal only when clear neuronal cell loss and/or gliosis was observed.

**Changes in the cytoarchitecture and patterns of immunostaining for GAD, PV and GFAP in cases not associated with brain tumours**

Relatively few cortical blocks showed abnormal patterns of immunostaining for GAD, PV and GFAP or abnormal cytoarchitecture (16%, 25%, 22% and 31%, respectively). However, if we consider the blocks in which at least one of the patterns of immunostaining or the cytoarchitecture was abnormal, then the majority (56%) of the blocks presented some kind of alteration. Furthermore, we must keep in mind...
that only a relatively small extent of cortex was analysed for each staining (on average four 100 μm thick sections per block were used for each immunocytochemical or thionin staining) and, therefore, there may be small regions with altered patterns (particularly patterns C and c) that might be not included in the analysis.

In conclusion, multiple, small, altered cortical regions seem to be a common characteristic of the epileptogenic neocortex in patients without brain tumours. Comparison of adjacent sections stained for PV and GAD, showed that there was a complete coincidence of normal patterns A and a, and altered patterns C and c for PV and GAD immunoreactivities, respectively, in the same regions. However, this correlation did not occur apparently in regions with patterns B and D of PV immunostaining, since a normal pattern of immunostaining for GAD was found in these regions.

**Changes in the cytoarchitecture and patterns of immunostaining for GAD, PV and GFAP in cases associated with brain tumours**

In the neocortex of epileptic patients with brain tumours, we found the same changes as in the cases not associated with brain tumours, i.e. regions showing altered patterns of immunostaining for GAD, PV and GFAP, focal cell loss and gliosis (Table 4). There was also a correlation between normal and abnormal patterns of immunostaining for GAD and PV (patterns A and a and patterns C and c, respectively). However, abnormal patterns of immunostaining were more frequently found in the cases associated with tumours than without tumours.

Since within each group of patients the 'primary' pathology associated with epilepsy varied (e.g. different types of tumours, neuronal cell loss or not in the hippocampus, etc.) and similar alterations in inhibitory neuronal circuits appear to occur in all cases, this suggests that there is possibly a common basic underlying mechanism that leads to seizure activity. In both tumoural and non-tumoural cases, among the inhibitory interneurons that are lost, chandelier cells appear to be one of the most affected type of cell and therefore they might play a crucial role in the aetiology of epilepsy (see below). Finally, it is possible that the degree of some of the pathological changes found in the hippocampus (or adjacent areas) might be related to the duration of symptoms (Mathern et al., 1995), but in the neocortex this was apparently unrelated.

**Possible significance of the immunocytochemical changes**

GABA is the neurotransmitter used by the majority of smooth nonpyramidal neurons (short-axon cells or interneurons). It is well-established that different types of GABAergic interneurons innervate both pyramidal cells and nonpyramidal cells (Houser et al., 1984). Among the interneurons that innervate pyramidal cells are chandelier cells and basket cells, which are immunoreactive for PV and are thought to play an important role in the control of pyramidal cell excitability (DeFelipe et al., 1989, 1993; Hendry et al., 1989; Blümcke et al., 1990; Lewis and Lund 1990; Ferrer et al., 1991; Hendrickson et al., 1991; Hof et al., 1991; Williams et al., 1992; del Río and DeFelipe, 1994). Co-localization immunocytochemical studies have shown that virtually all PV-positive cells are also immunoreactive for GABA and that a large proportion of GABA cells also display immunoreactivity for PV (for review, see DeFelipe, 1993). Thus a decrease in GABA neurons in a given cortical region could lead to a general reduction of inhibition in all kinds of neurons (pyramidal and nonpyramidal) located in that region, whereas a decrease in PV immunoreactive neurons could lead to a more selective although important reduction in the inhibitory synaptic control on pyramidal cells.

It has been shown that expression in cortical neurons of a variety of transmitters or transmitter-related substances (including GAD and PV) can be altered in an activity-dependent manner (Hendry and Jones, 1986, 1988; Blümcke et al., 1994; for a review see Jones, 1993). Thus, it is possible that the decrease in GAD and PV immunoreactivities in the epileptogenic neocortex could be due to activity-dependent fluctuations in the immunocytochemical staining, and that these changes are reversible and dynamic. In a previous study of similar material (DeFelipe et al., 1993), cortical regions showing decreased immunostaining for PV were examined (in particular cortical regions with pattern C) at the electron microscope level. In these regions cell death (apoptotic bodies) and an apparent decrease in axon terminals forming symmetric synapses were found. In the present study, regions showing patterns C and c of immunostaining for PV and GAD, respectively, presented a clear cell loss and this loss affected nonpyramidal cells but also pyramidal cells in a variable degree. Immunocytochemical studies with antibodies to PV and to the glutamate receptor subunits GluR2/3 and GluR5/6/7 of the δ/γ-aminobutyric acid, 5-methyl-4-isoxazole propionic acid/kainate and kainate receptor subtypes have shown that in the human temporal neocortex removed from epileptic patients the majority of small patches of decreased immunoreactivity for PV (pattern C) are coincident with patches of decreased GluR2/3 and GluR5/6/7 immunostaining (DeFelipe et al., 1994). Most GluR2/3 and GluR5/6/7 immunoreactive cells are pyramidal cells, and receptor subunit immunoreactivity is localized, in part, to asymmetric postsynaptic densities (Huntley et al., 1994). Thus, these regions of decreased GluR2/3 and GluR5/6/7 subunit immunoreactivity may also represent a disorganization of pyramidal cell-mediated excitatory synaptic circuitry (DeFelipe et al., 1994). Further immunocytochemical studies (DeFelipe et al., 1996) indicate that the lighter staining of pyramidal cells within these patches of decreased receptor subunit immunostaining probably represents cells undergoing a general decrease in protein synthesis or an increase in protein degradation.
Whether these cells are damaged and will eventually die, or whether these changes are reversible awaits further studies.

We have recently used quantitative electron microscope methods to compare the synaptic density in the neuropil between regions with a normal pattern of immunostaining for PV (pattern A) and regions with abnormal patterns of immunostaining for PV showing a pattern B (with normal cytoarchitecture) or showing pattern C (with neuronal cell loss) in the temporal cortex of some epileptic patients examined in the present study (P. Marco and J. DeFelipe, unpublished observations). We found that the overall synaptic density in regions with patterns B and C was significantly higher than that in regions with pattern A. In addition, the density of presumptive excitatory (asymmetric) synapses was significantly higher in regions with pattern B and C as compared to regions with pattern A. However, the density of presumptive inhibitory (symmetric) synapses only changed in regions with pattern C where a significantly lower density was found when compared to that in regions with pattern A or B. The fact that regions showing pattern B (and D) of decreased immunostaining for PV apparently present a normal cytoarchitecture and normal pattern of staining for GAD, is consistent with the finding that the density of symmetric synapses in the neuropil do not change in regions with pattern B. Thus, it is possible that in these cortical regions, there is a selective loss of chandelier and basket cells. Since nonpyramidal cells are relatively few and chandelier and basket cells represent only a minor portion of the total population of nonpyramidal cells, the loss of these neurons may pass unnoticed in thionin-stained sections. However, we have not yet examined under the electron microscope the axon initial segments and somata of pyramidal cells in cortical regions with patterns B and D to determine whether there is a loss of immunoreactivity or whether the axon terminals of these cells (axo-axonic and axo-somatic terminals, respectively) have been lost. Thus, in the human epileptic neocortex there are permanent changes in cortical circuits but transient changes may also occur.

**Correlation of intraoperative ECOG with cytoarchitecture and patterns of immunostaining for GAD, PV and GFAP**

Comparison of the intraoperative ECOG characteristics of a given cortical region with the cytoarchitectural characteristics or patterns of immunostaining for PV, GAD or GFAP displayed in that region showed a lack of correlation between ECOG findings and the other parameters examined, confirming previous studies using similar material (DeFelipe et al., 1993, 1994). Similarly, the electrophysiological properties of human neocortical neurons recorded in the deep layers of neocortical slices of the temporal and frontal lobe maintained in vitro that had been removed from epileptic patients for the relief of intractable epilepsy, did not vary with the degree of epileptiform activity recorded in situ at surgery (Avoli and Olivier, 1989).

As previously discussed (DeFelipe et al., 1994), the lack of correlation between the anatomical and physiological findings makes it difficult to know how or whether epileptiform activity leads to alterations of cortical circuits or, conversely, how or whether these abnormal circuits contribute to epileptiform activity. However, it cannot be disregarded that regions with abnormal cortical circuits and normal ECOG at the time of surgery had epileptiform activity at some other time, or that abnormal activity in an anatomically normal region could be the result of a projected activity from a primary epileptogenic focus. Furthermore, the occurrence and degree of spiking activity detected by ECOG at any given cortical region can be modified under various surgical conditions (e.g. type and levels of anaesthesia, pharmacological activation, etc.). The latter and other observations makes the value of the use of interictal epileptic discharges recorded by ECOG to guide cortical resections controversial (Ojemann, 1992). Our results also question the value of ECOG to determine the excision limit, since non-spiking activity is commonly found in regions that show changes in cortical circuits.

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