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REVIEW ARTICLE

Chandelier cells and epilepsy

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Summary

The main goal of this article is to review certain aspects of the circuitry of the human cerebral cortex that may be particularly relevant for the development, maintenance or spread of seizures. There are a number of different structural abnormalities that are commonly found in the cortex of epileptic patients, but these abnormalities do not appear to be intrinsically epileptogenic, since some patients displaying them are epileptic (after variable delays) whereas others are not. Therefore, cortical circuits in an affected brain may undergo a series of changes that finally cause epilepsy. In this article, it is proposed that the chandelier cell, which is considered to be the most powerful cortical GABAergic inhibitory interneuron, is probably a key component of cortical circuits in the establishment of human intractable temporal lobe epilepsy. These cells (among other types) have been found to be lost or reduced at epileptic foci in both experimental animals and epileptic patients. A hypothesis is presented by which the normal variability in the number of interneurons might explain the predisposition of some individuals to develop epilepsy more than others as a result of a lesion or other precipitating factors that lead to loss of neurons. The sources of GABAergic input on dendrites and somata of cortical pyramidal cells originate from many and diverse types of interneurons but, at the level of the axon initial segment of these cells, all synapses come from a few chandelier cells (five or less). Loss of one class of interneurons ending on soma and dendrites might have relatively little impact on the inhibitory control of the pyramidal cell. However, if chandelier cells were affected, it would have serious consequences for the inhibitory control of the pyramidal cells. Evidence suggests that the loss of chandelier cells may be non-specific and that when this occurs epilepsy may develop. Therefore, these cells might represent a key component in the aetiology of human temporal lobe epilepsy.

Keywords: human; cortical circuitry; interneurons; parvalbumin; neuronal loss

Abbrevation: Ch terminal = terminal portion of the axon of chandelier cells

Introduction

Epilepsy frequently is associated with a variety of brain lesions, tumours or other structural abnormalities which mostly affect the cerebral cortex. However, these alterations are not intrinsically epileptogenic, since some patients are epileptic, whereas others with similar abnormalities are not, or they develop epilepsy after a variable delay. One of the best examples is that of brain tumours, which may or may not cause epilepsy, even when they are of the same type and located in the same brain area. Furthermore, seizures associated with brain tumours or head injuries may appear relatively soon or after a long 'latent' period that may extend for several years (Ketz, 1974; Spencer *et al*., 1984; Morrell, 1989; Dinner, 1993; Morris and Estes, 1993). Thus, it seems that cortical tissue in the altered brain suffers a series of changes that eventually lead to epilepsy.

Temporal lobe epilepsy is one of the most frequent types of human epilepsy, and a variety of surgical procedures have been developed for the treatment of intractable temporal lobe epilepsy (e.g. Falconer, 1971*a*; Wieser, 1996). Resected tissue can be examined successfully using a variety of sophisticated biochemical and anatomical techniques for investigating the mechanisms underlying epilepsy. However, the basic mechanism by which seizure activity is related to abnormal cortical circuits is still under debate (Ben-Ari and Represa, 1990; Sloviter, 1991; McNamara, 1994; Schwartzkroin, 1994*a*). Most hypotheses are based on alterations of glutamatergic (excitatory) and GABAergic (inhibitory) cortical neuronal systems in either the hippocampus, the neocortex or both (Lloyd *et al*., 1986; Prince and Connors, 1986; Sherwin and van Gelder, 1986; Houser, 1991; Ribak,

1991; Avanzini *et al*., 1992; Meldrum, 1995; DeFelipe *et al*., 1996). However, some researchers (including my group) maintain that, in spite of the variety of brain abnormalities associated with epilepsy, it is possible that a common basic underlying mechanism exists. In this work, I shall propose that a particular type of interneuron, the chandelier cell, is probably a key component of cortical circuits in the establishment of human epilepsy. The following presentation will be focused mainly on the human neocortex in temporal lobe epilepsy, unless otherwise specified.

Types of neocortical neurons

The neocortex contains two major classes of neurons: spiny neurons and smooth non-pyramidal neurons. Spiny neurons are represented by pyramidal cells and spiny non-pyramidal cells. Pyramidal cells are the projection neurons, they are present in all layers except layer I, and they are the most abundant (~70–85% of the total neuron population). Spiny non-pyramidal cells are interneurons confined to the middle layers of the cortex (principally layer IV), the most typical being the spiny stellate cell (e.g. Fairén *et al*., 1984; Lund, 1984). Smooth non-pyramidal neurons are also interneurons, but with smooth or sparsely spiny dendrites. Pyramidal and spiny stellate cells are excitatory and are likely to use glutamate and/or aspartate, whereas the majority of smooth interneurons are likely to use GABA and, thus, are inhibitory (for review, see Gilbert, 1983; Houser *et al*., 1984; Ottersen and Storm-Mathisen, 1984; Streit, 1984; White, 1989; Conti, 1991; DeFelipe and Fariñas, 1992; DeFelipe, 1993; Jones, 1993; Somogyi *et al*., 1998). In this presentation, I will refer to smooth non-pyramidal neurons as aspiny interneurons. These interneurons are found in all cortical areas and layers and make up ~15–30% of the total neuron population. These interneurons form a morphologically heterogeneous group, and different morphological types are recognized mainly on the basis of their patterns of axonal arborization (e.g. Jones, 1975; Fairén *et al*., 1984).

What is a chandelier cell?

The chandelier cell is a type of aspiny interneuron with smooth dendrites and is distinguished by the terminal portions of its axon that form short vertical rows of boutons (Ch terminals) resembling candlesticks (Szentágothai and Arbib, 1974) (Fig. 1). These cells have been found not only in the neocortex, but also in several areas of the paleocortex, archicortex and mesocortex, which include the piriform cortex, entorhinal cortex, subiculum, hippocampus, fascia dentata and cingulate cortex (Szentágothai and Arbib, 1974; Jones, 1975; Fairén and Valverde, 1980; Somogyi *et al*., 1982, 1983*b*, 1985; Soriano and Frotscher, 1989; Soriano *et al*., 1990, 1993; Schmidt *et al*., 1993; Buhl *et al*., 1994; Gabbott and Bacon, 1996; Martínez *et al*., 1996; Mikkonen *et al*., 1997). In general, the chemical and morphological characteristics of these cells are similar in all divisions of the cerebral cortex, but in the present work I shall deal only with chandelier cells of the neocortex, unless otherwise specified.

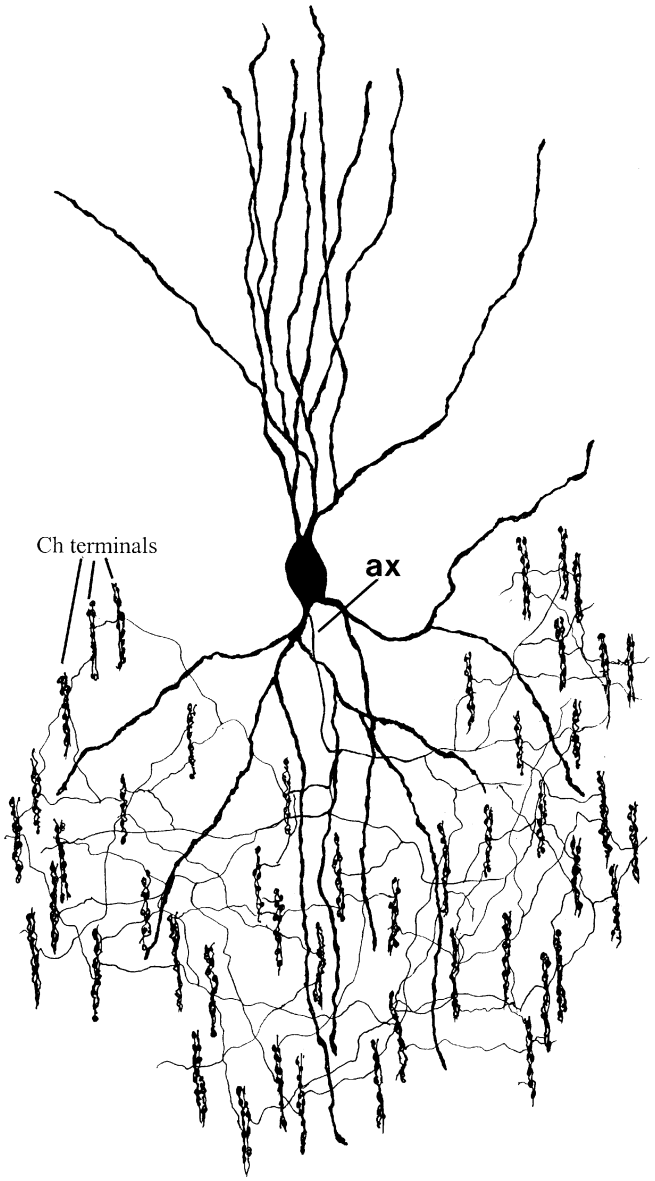


Fig. 1 Schematic drawing of a chandelier cell based on the immunostaining of chandelier cells with parvalbumin immunocytochemistry in the human neocortex. This cell is distinguished by the characteristic terminal portions of its axon, which form short vertical rows of boutons (Ch terminals) resembling candlesticks. Each Ch terminal innervates a single axon initial segment of a pyramidal cell. ax indicates the initial portion of the axon, which originates from a dendrite.

These cells, or the Ch terminals, have been visualized using a variety of methods: the Golgi method (e.g. Jones, 1975; Szentágothai, 1975; Somogyi, 1977; Fairén and Valverde, 1980; Peters *et al*., 1982; Somogyi *et al*., 1982; DeFelipe *et al*., 1985; Kisvárday *et al*., 1986; Marin-Padilla, 1987; Lewis and Lund, 1990); immunocytochemical methods using antibodies directed against parvalbumin (DeFelipe

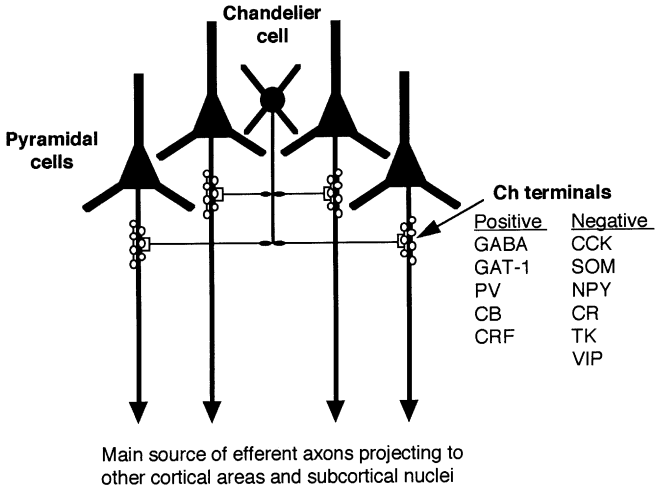


Fig. 2 Schematic diagram illustrating chemical characteristics and synaptic connections of chandelier cells with pyramidal cells. The latter cells are the main source of cortical efferent axons and of intracortical collaterals and, therefore, responsible for spreading epileptiform activity. On the basis of immunocytochemistry, chandelier cells can be defined chemically as GABAergic cells that show a high level of expression of the GABA transporter GAT-1 in their Ch terminals and that contain the calcium-binding proteins parvalbumin (PV) and calbindin (CB), but not calretinin (CR). Some chandelier cells also contain the neuropeptide corticotropin-releasing factor (CRF), but not other neuropeptides, namely cholecystokinin (CCK), somatostatin (SOM), neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP) and tachykinins (TK). Chandelier cells are chemically heterogeneous, since, depending on the species, cortical areas and layers, chandelier cells are immunoreactive for only selected combinations of these substances with GABA (see text for further details).

et al., 1989; Lewis and Lund, 1990; Williams *et al*., 1992; Condé *et al*., 1994; del Río and DeFelipe, 1994; Anderson *et al*., 1995), against calbindin (del Río and DeFelipe, 1997), against corticotropin-releasing factor (Lewis *et al*., 1989; Lewis and Lund, 1990) or against GABA transporter GAT-1 (DeFelipe and González-Albo, 1998; Woo *et al*., 1998); by intracellular injections of horseradish peroxidase (Freund *et al*., 1983) or Lucifer Yellow (Naegele and Katz, 1990); and by intracellular staining with biocytin (Kawaguchi, 1995; Kawaguchi and Kubota, 1998).

Studies using immunocytochemistry for GABA or its synthesizing enzyme glutamic acid decarboxylase showed that chandelier cells are GABAergic and, thus, inhibitory (Peters *et al*., 1982, Freund *et al*., 1983; Hendry *et al*., 1983, DeFelipe *et al*., 1985; Somogyi *et al*., 1985; Buhl *et al*., 1994). On the basis of immunocytochemistry, chandelier cells may be chemically defined as GABAergic cells which contain the calcium-binding proteins parvalbumin and calbindin, but not calretinin, and which contain the neuropeptide corticotropin-releasing factor, but not other neuropeptides, namely cholecystokinin, somatostatin, neuropeptide Y, vasoactive intestinal polypeptide and tachykinins (for a review, see DeFelipe and Fariñas, 1992) (Fig. 2). However, these cells are chemically heterogeneous, since, depending on the species, cortical areas and layers, chandelier cells are either immunoreactive or not for selected combinations of these substances (see Lewis and Lund, 1990; del Río and DeFelipe, 1994, 1997; and references therein). For example, using immunocytochemistry for corticotropin-releasing factor to examine the prefrontal and occipital cortex of *Macaca mulatta*, *Macaca fascicularis* and *Saimiri sciureus*, Lewis and Lund found that Ch terminals were labelled only in the squirrel monkey (*S. sciureus*), mainly in layer IV of the prefrontal cortex (Lewis and Lund, 1990). Calbindin immunocytochemistry labels a small subpopulation of Ch terminals located mainly in layers V and VI of the human neocortex, but not in other species (del Río and DeFelipe, 1997). Nevertheless, the most general, prominent and complete staining of Ch terminals is obtained with immunocytochemistry for parvalbumin (Figs 2 and 3) and GAT-1 (DeFelipe and González-Albo, 1998).

The axon terminals that make up Ch terminals have been shown to innervate only the axon initial segments of pyramidal cells on which they form symmetrical synapses (e.g. Somogyi, 1977; Fairén and Valverde, 1980; Peters *et al*., 1982; Somogyi *et al*., 1982; Freund *et al*., 1983; DeFelipe *et al*., 1985, 1993; del Río and DeFelipe, 1994). Chandelier cells are the major or sole source of synapses on pyramidal cell axon initial segments, and each single chandelier cell axon can give off several hundred Ch terminals (e.g. Freund *et al*., 1983; DeFelipe *et al*., 1985). Each Ch terminal innervates a single axon initial segment. However, a single axon initial segment may be innervated by one or a few Ch terminals (five or less) which, in turn, may originate from the same or different chandelier cells (Fairén and Valverde, 1980; Peters *et al*., 1982; Freund *et al*., 1983; Somogyi *et al*., 1983*c*; reviewed in Somogyi *et al*., 1982; DeFelipe and Fariñas, 1992).

Finally, because these cells are inhibitory and ubiquitously distributed and because the axon initial segments of pyramidal cells appear to be strategically important regions for the control of pyramidal cell excitability (Douglas and Martin, 1990; Stuart and Sakmann, 1994), chandelier cells are considered to be the most powerful cortical inhibitory interneuron. Because pyramidal cells are the main source of cortical efferent axons and of intracortical collaterals, they are responsible for the spread of epileptiform activity; this makes chandelier cells particularly relevant in epilepsy, as originally proposed by Somogyi *et al*. and then by Ribak and by Kisvárday *et al*. (Somogyi *et al*., 1983*b*; Ribak, 1985; Kisvárday *et al*., 1986; for a review, see Ribak, 1991).

Surgical treatment of temporal lobe epilepsy and pathological findings

Neuronal loss

Neuronal loss (and gliosis) is characteristic of epileptogenic tissue; however, a comprehensive review of the current voluminous literature about the relationship between neuronal loss and epilepsy is beyond the scope of this presentation.

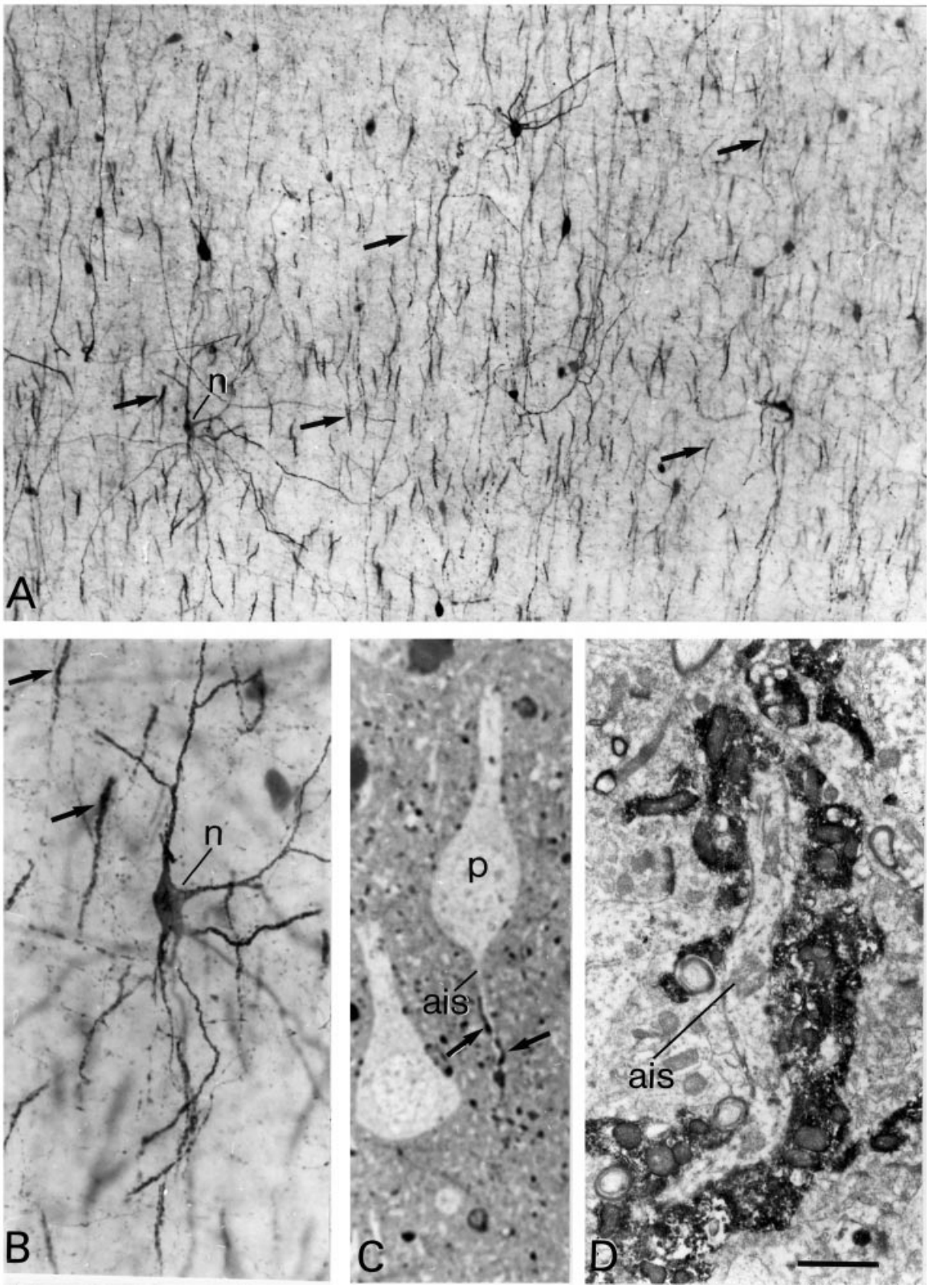


Fig. 3 (**A**) Parvalbumin immunostaining through the normal human middle temporal gyrus. Photomicrograph extending through layer VI, illustrating numerous parvalbumin-immunoreactive chandelier cell axon terminals (Ch terminals), some of which are indicated by arrows. Scale bar: 100 μm. (**B**) Higher magnification of the immunoreactive neuron (n) shown in **A**. Arrows indicate some Ch terminals. Scale bar: 13 μm. (**C**) Photomicrograph of a semi-thin (2 μm thick) plastic section after counterstaining with 1% toluidine blue in 1% borax, showing a parvalbumin-immunoreactive Ch terminal (arrows) outlining the axon initial segment (ais) of a pyramidal cell (p). Scale bar: 15 μm. (**D**) Electron micrograph showing an axon initial segment (ais) surrounded by immunoreactive boutons of a parvalbumin-immunoreactive Ch terminal. Scale bar: 1 μm. (**A** and **B** are from del Río and DeFelipe, 1997; **D** is from del Río and DeFelipe, 1994.)

In patients with symptomatic epilepsy, neuronal loss occurs irrespective of the primary cause of the lesion (e.g. underlying tumour, infection, trauma, vascular accidents) (Meldrum and Bruton, 1992). In cases not associated with brain tumours and without obvious pathological defects, standard histopathological examination of resected brain tissue reveals a range of findings that vary from severe neuronal loss to no loss at all (Meldrum and Bruton, 1992). The most typical alteration observed in patients with temporal lobe epilepsy is neuronal loss and gliosis in the hippocampus (hippocampal or Ammon's horn sclerosis) (Margerison and Corsellis, 1966; Falconer, 1974; Babb and Brown, 1987; Meldrum and Bruton, 1992; Babb and Pretorius, 1993), although the neocortex of the anterolateral superior, middle and inferior temporal gyri (to which I shall refer collectively as temporal neocortex) frequently is found to be normal (Babb *et al*., 1984). In these patients, MRI may be normal or may show hippocampal atrophy or abnormal signals (Cascino *et al*., 1991; Jackson *et al*., 1994). This is in line with the well-known fundamental role attributed to the hippocampus in human temporal lobe epilepsy (Schwartzkroin, 1994*b*), which is often considered (together with the amygdala) as 'the epileptogenic zone'. However, significant interictal activity arising from the temporal neocortex and extrahippocampal mesial temporal structures frequently is observed (including cases with hippocampal sclerosis). Furthermore, due to the strong reciprocal connections between mesial temporal structures and the temporal neocortex (Van Hoesen, 1982; Suzuki and Amaral, 1994), it is often difficult to ascertain whether spiking activity recorded from a given electrode is primarily a projected or an intrinsic activity (e.g. Walczak, 1995; O'Brien *et al*., 1996). The experience of many surgeons indicates that, in general, a better result is obtained from a large resection compared with small and restricted removals. Thus, the most common surgical procedure for temporal lobe epilepsy is anterior temporal lobectomy, which consists of the ipsilateral removal of the anterolateral temporal cortex, the amygdala and the anterior portion of the hippocampus and adjacent cortex (Olivier, 1992).

Selective amygdalohippocampectomy (removal of the amygdala, the anterior hippocampus and part of the parahippocampal gyrus) is an alternative surgical procedure (Wieser and Yasargil, 1982) which, in principle, would be the most appropriate for surgical treatment when sclerosis of the hippocampus is the most probable lesion. Nevertheless, this surgical procedure is applied only to a relatively few patients in whom seizure onset is clearly restricted to mesial temporal structures; but even in these cases, although the amygdalohippocampectomy is highly effective in some patients, there is no guarantee of a good surgical outcome in all patients (see Wieser, 1991, 1996; Mayanagi *et al*., 1996; Alarcon *et al*., 1997). Furthermore, as pointed out by Babb and Brown (Babb and Brown, 1987), because of the occurrence of extrahippocampal pathologies in some cases with hippocampal sclerosis (dual pathology), amygdalohippocampectomy should be a less successful surgical technique than anterior temporal lobectomy, which would remove both hippocampal and extrahippocampal lesions. For example, in a series of 178 epileptic patients who underwent *en bloc* temporal lobectomies (reviewed by Lévesque *et al*., 1991), dual pathology was present in 30% of the cases (see also Margerison and Corsellis, 1966). Additionally, although there are a number of clinical and electrophysiological differences between temporal lobe epileptic patients presenting mesial temporal sclerosis and those with discrete temporal neocortical lesions (Saygi *et al*., 1994; O'Brien *et al*., 1996; Gil-Nagel and Risinger, 1997), in practice these differences are not sufficient to establish such a distinction in an individual patient (see O'Brien *et al*., 1996). Finally, Jones-Gotman *et al*. have shown that selective, minimal removals of temporal lobe structures do not preserve certain intellectual capabilities better than larger resections, since neocorticectomies, selective amygdalohippocampectomies and anterior temporal lobectomies led to similar neuropsychological deficits (regarding learning and memory for verbal and visuospatial material) (Jones-Gotman *et al*., 1997). Thus, many surgeons are inclined toward anterior temporal lobectomy as the surgical procedure to be used.

In a recent study made by Alarcon *et al*. on the relationship between patterns of discharge propagation during acute electrocorticography and surgical treatment of temporal lobe epilepsy, a very significant association between a poor outcome and the retaining of regions where discharges showed earliest peaks ('leading regions') was found (Alarcon *et al*., 1997). Therefore, these authors concluded that leading regions were potentially epileptogenic regions that should be removed in order to improve seizure control. Within a given patient, multiple leading regions, which were not necessarily associated with the anatomically pathological region, were found. Interestingly, in the 23 cases with mesial temporal sclerosis studied by Alarcon and colleagues, the main leading regions were localized in the hippocampus in only 43% of the patients, whereas in the remaining 57% these leading regions were localized either in the temporal neocortex or in the extrahippocampal basal temporal cortex. These results support previous studies which emphasized the contribution of the neocortex to seizure development and propagation. However, the temporal neocortex was found by these authors to be normal on the basis of standard histopathological methods, which is the most common finding in the resected temporal neocortex (see above). Therefore, it is generally thought that the temporal neocortex without any apparent structural abnormality may be epileptogenic due to unknown mechanisms and that this may occur irrespective of the primary pathology found in the resected tissue.

Loss of interneurons

Three long-continuing questions still prevail in the field of epilepsy pathology: first, how and when neuronal loss occurs; secondly, whether all types of neurons are equally affected; and thirdly, whether this loss is secondary to epileptic

seizures or represents a primary process which initiates the development of seizure activity.

Standard histopathological techniques do not usually detect subtle changes in neuronal circuitry, especially if particular types of interneurons are selectively affected, because they represent a minor population of neurons whose loss may pass unnoticed. Along these lines, in certain experimental animal models of focal epilepsy, it has been shown, using immunocytochemical and electron microscope techniques, that in the epileptogenic neocortex there is a preferential loss of GABAergic neurons and axon terminals; two of the GABAergic cell types that are lost at epileptic foci are the basket cell and chandelier cell (Ribak *et al*., 1979, 1982; Ribak, 1985; Houser *et al*., 1986; for reviews, see Houser, 1991; Ribak 1991). Loss of inhibition at the epileptic foci would cause epileptic activity. Finally, Williams *et al*. found, in a patient showing generalized slow and paroxysmal activity recorded by EEG and diagnosed with neuronal ceroid-lipofuscinosis (Batten disease), but with no evidence of neuronal degeneration or loss, that the perikarya, axon hillock and axon initial segment of neocortical pyramidal cells were surrounded by reactive processes of astrocytes and possessed few or no inhibitory synapses at these sites, whereas presumptive excitatory synapses were abundant in the neuropil (Williams *et al*., 1977). These authors suggested that the loss of the inhibitory synapses might be the cause of the paroxysmal activity.

Parvalbumin immunostaining as a tool for identifying abnormal circuits

Since parvalbumin immunocytochemistry labels a sub-population of GABAergic interneurons (which include chandelier cells and basket cells) (Celio, 1986; DeFelipe *et al*., 1989; Hendry *et al*., 1989; Lewis and Lund, 1990; Andressen *et al*., 1993), we used this staining to label these cells in cortical tissue removed from epileptic patients (Figs 4–6). The aim of these studies (DeFelipe *et al*., 1993; Marco *et al*., 1996, 1997) was mainly to investigate whether chandelier cells and basket cells were lost from sections of neocortex from patients with temporal lobe epilepsy, while adjacent sections showed normal appearances in standard histopathological preparations. The examination was independent of whether or not the condition was associated with brain tumours or presented neuronal cell loss in the hippocampus. In these studies, multiple small regions or patches (often 200 μm to 1 mm wide) showing variable degrees of decreased parvalbumin immunostaining commonly were observed (see also Ferrer *et al*., 1994; Spreafico *et al*., 1998), and were independent of the primary pathological findings (Figs 4–6). Since no patches of decreased parvalbumin immunostaining were observed in sections from neurologically normal human neocortical tissue (Marco *et al*., 1996; Marco and DeFelipe, 1997), we assume that these changes are probably related to the occurrence of seizures in the brains of these patients (see below).

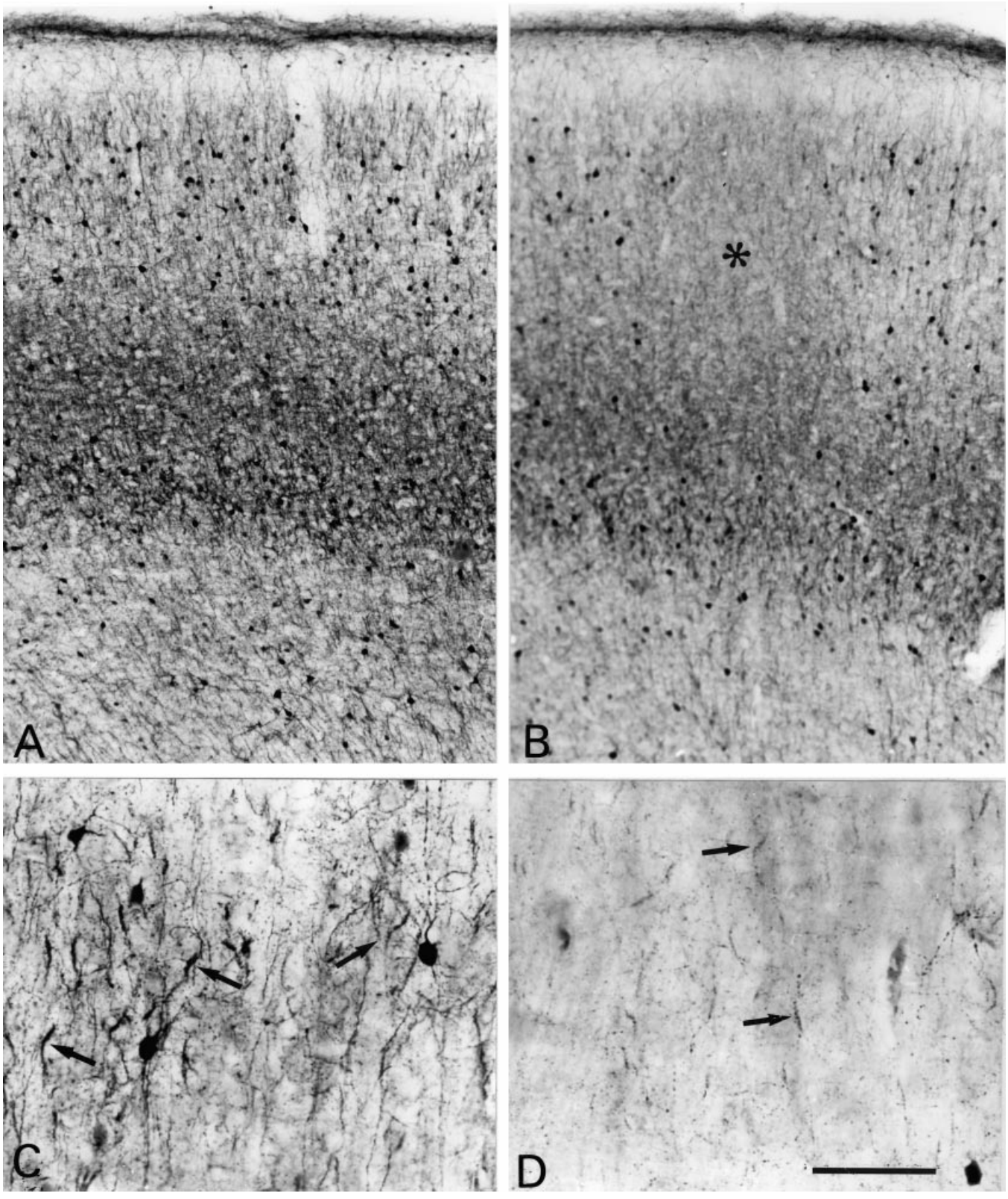
For simplicity, I shall refer to all abnormal patterns of parvalbumin immunostaining collectively as 'decreased parvalbumin immunostaining', unless otherwise specified. The decrease apparently always affected chandelier cell axon terminals and, to a greater or lesser extent, other types of parvalbumin-positive neurons, including basket cell axon terminals. For example, there were regions showing a pattern of parvalbumin immunostaining that, at first glance, was indistinguishable from the normal pattern of immunostaining, but closer inspection showed a clear decrease or a virtual lack of chandelier cell axon terminals (Fig. 6B), whereas in other regions all types of parvalbumin-positive cells including chandelier cells appeared to be affected to various degrees (Marco *et al*., 1996, 1997) (Fig. 5A and B). Nevertheless, there are still always relatively numerous terminals in the neuropil and around the somata of pyramidal cells in the regions with decreased parvalbumin immunostaining, whereas the decrease in chandelier cell axon terminals appears to be complete, because vertical rows of boutons, which are typical of chandelier cell axon terminals, are not stained (Fig. 7).

Examination of Nissl-stained sections adjacent to those showing decreased parvalbumin immunostaining suggested that the cortical tissue was apparently normal in most cases, but no quantitative analyses were performed. However, regions showing the so-called pattern C (which consists of a virtual lack of parvalbumin-immunoreactive neurons and a reduction in parvalbumin-immunoreactive processes, affecting mainly chandelier cell axon terminals) were associated with focal cell loss (Marco *et al*., 1996). This focal cell loss consisted of a moderate or severe decrease in neurons that affected (at least in the severe cases) both pyramidal cells

Fig. 4 (**A** and **B**) Parvalbumin immunostaining through layers I–V of the human middle temporal gyrus, showing a normal pattern (**A**) and an abnormal pattern (**B**) of immunostaining. Note the dense immunoreactive band located in the middle portion of the cortex, which is one of the characteristic features of parvalbumin immunostaining in the human cortex. In **B** is shown a small, focal decrease of immunostaining (asterisk) just above the immunoreactive band of the middle cortical layers. This photograph was taken from an epileptic patient in whom neuropathological examination of the hippocampus and parahippocampal gyrus showed gliosis in the parahippocampal gyrus. (**C**) Photomicrograph from layer IV of the normal cortex (superior temporal gyrus), showing numerous parvalbumin-immunoreactive Ch terminals (arrows). (**D**) Photomicrograph from layer IV from a cortical region showing a focal decrease in parvalbumin immunostaining in the superior temporal gyrus. In this region, fewer immunoreactive elements, in general, are evident (arrows indicate some Ch terminals) compared with the normal cortex (**C**). This photomicrograph was taken from a patient in whom the neuropathological finding was a cholesteatoma adjacent to the amygdala. Scale bars: 312 μm for **A** and **B** and 100 μm for **C** and **D**. (**A** and **B** are from Marco *et al*., 1996; **C** and **D** are from DeFelipe *et al*., 1993.)

and GABAergic interneurons. Frequently, these regions were so small that they were very difficult to identify in Nissl-stained sections. However, once a region with pattern C of parvalbumin immunostaining was detected, then a decrease in cells was readily identified in the adjacent Nissl-stained sections. This correlation was demonstrated more directly after examination of 2 μm semi-thin plastic sections from patches of decreased immunostaining in material prepared for electron microscopy (Marco and DeFelipe, 1997).

It should be mentioned that patches of decreased



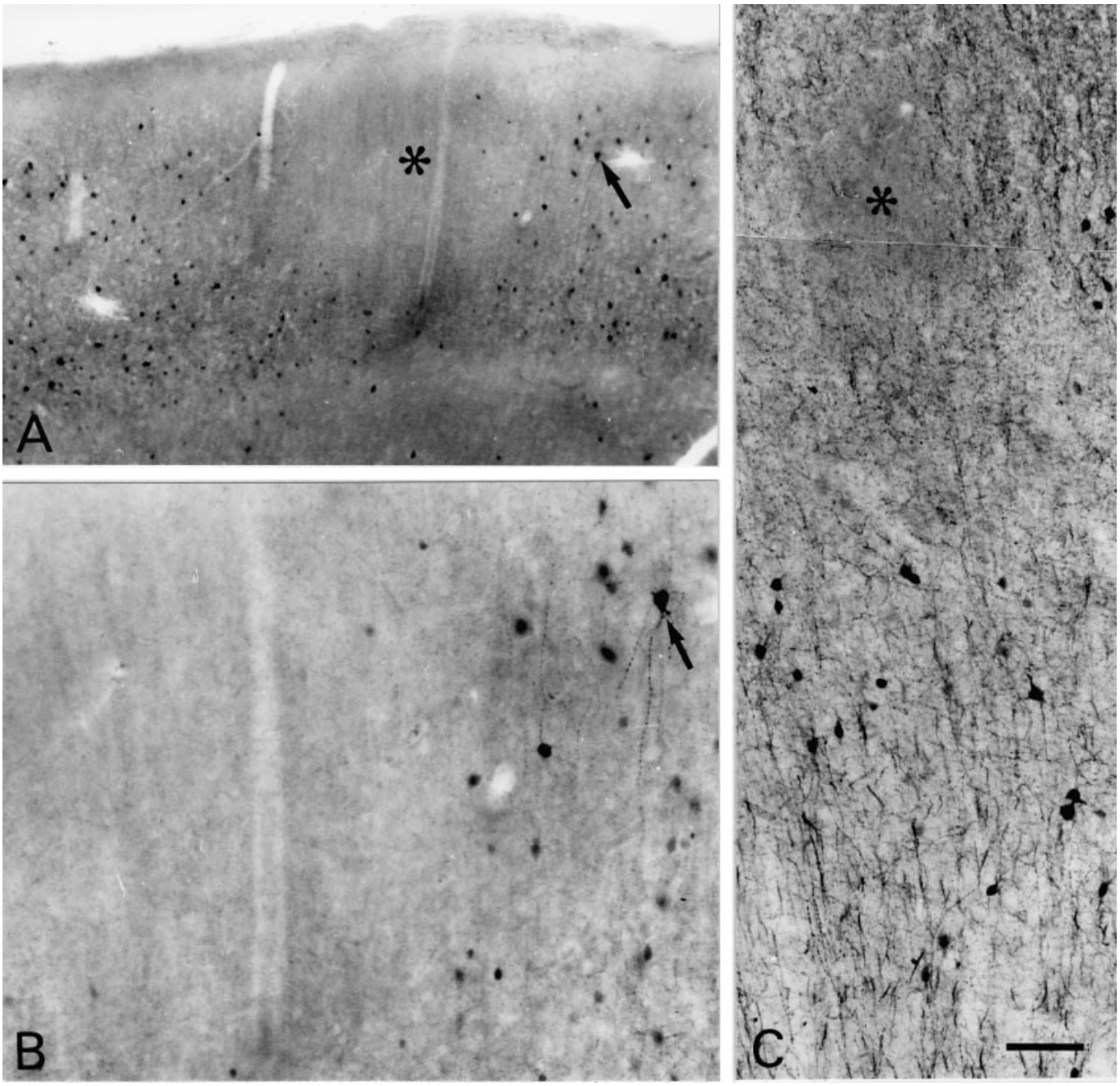


Fig. 5 Photomicrographs illustrating decreased parvalbumin immunostaining (asterisks) in relatively large (**A**) and small (**C**) cortical regions from the middle temporal gyrus from two different patients. **B** is a higher magnification of **A**, showing the same neuron (arrow) as in (**A**). Note the considerable decrease in immunoreactivity in **A** and **B** compared with **C**. The photomicrographs in **A** and **B** were taken from an epileptic patient in whom the neuropathological findings in the hippocampus and parahippocampal gyrus were neuonal loss and gliosis in both regions, whereas **C** was taken from an epileptic patient in whom no significant alterations were found in the parahippocampal gyrus (the hippocampus was not analysed). Scale bars: 237 μm for **A**; 92 μm for **B**; and 100 μm for **C**. (**A** and **B** from DeFelipe *et al*., 1994; **C** from Marco and DeFelipe, 1997.)

parvalbumin immunostaining were not found in all patients. As pointed out previously (Marco *et al*., 1996), only a relatively small amount of neocortex was analysed using parvalbumin immunocytochemistry in each patient and, therefore, there may be small regions with altered patterns that might not be included in the analysis. In this regard, it is important to note that after post-mortem pathological examination of the brains of epileptic patients, patchy neuronal loss was found in the frontal cortex in some cases with hippocampal sclerosis (Margerison and Corsellis, 1966). Thus, neocortical alterations may be more widespread than previously thought.

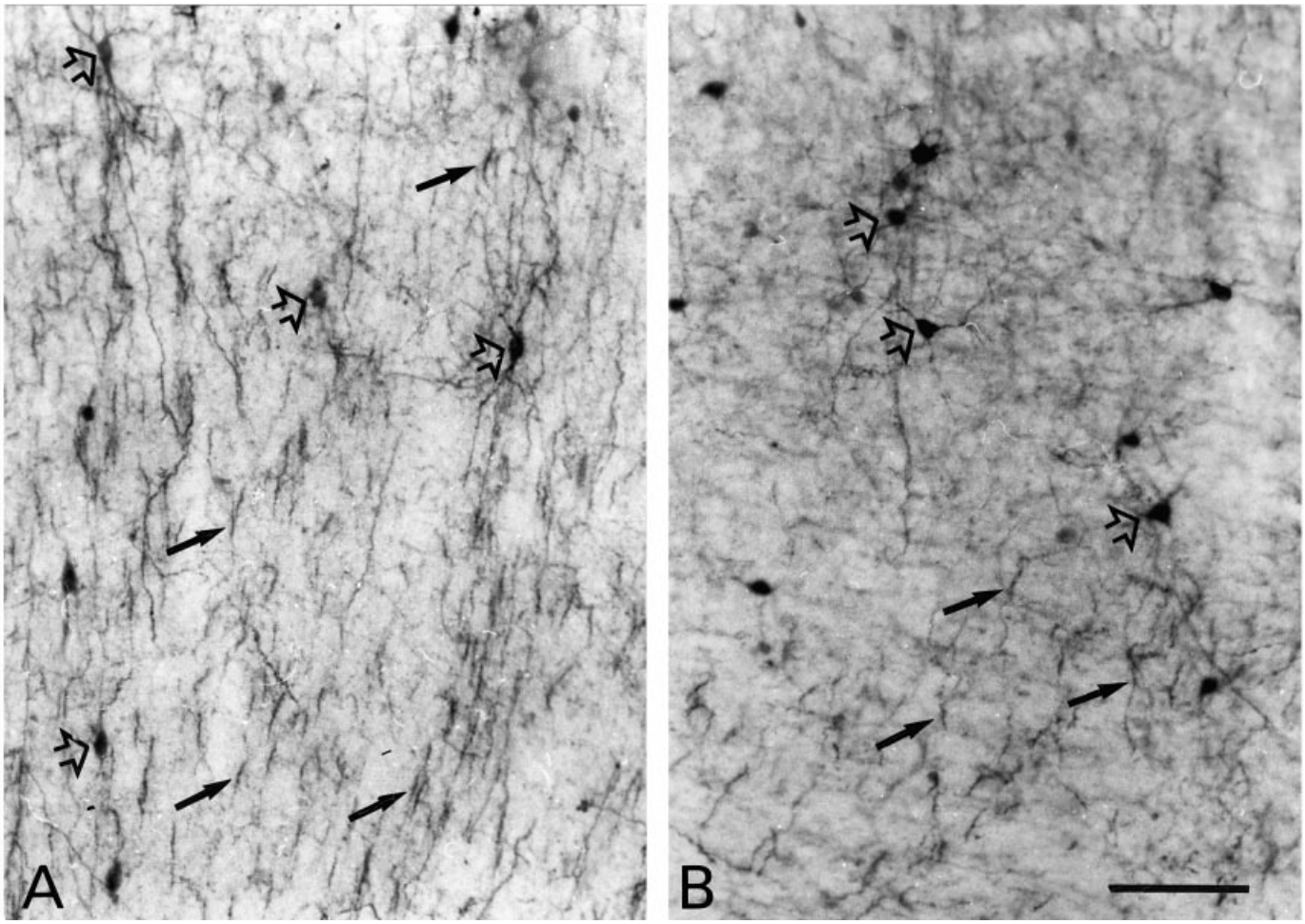


Fig. 6 Parvalbumin immunostaining through the middle temporal gyrus from an epileptic patient who presented a hamartous lesion with a mixed population of ganglion cells and glial cells with meningeal affectation in the temporal lateral neocortex. Photomicrographs through layer VI from two adjacent regions located at a distance of ~1 mm. Some parvalbumin-immunoreactive chandelier cell axon terminals (Ch terminals) are indicated by arrows. **A** was taken from a peritumoural region showing a normal cytoarchitecture in Nissl-stained sections and normal pattern of immunostaining, whereas **B** was taken at ~2 mm from the tumour, and showed gliosis and a clear decrease in immunostaining affecting mainly the Ch terminals. Open arrows indicate immunoreactive neurons. Scale bar: 100 μm.

Decreased parvalbumin immunostaining and electrocorticography

Patches of decreased parvalbumin immunostaining were found, surprisingly, in both spiking and non-spiking cortex, as revealed by intraoperative electrocorticography, making it difficult to correlate unambiguously abnormal parvalbumin immunostaining with patterns of spiking cortex (see Marco *et al*., 1996). Similarly, Ferrer *et al*. found multiple patches of decreased parvalbumin (and calbindin) immunoreactivity in the neocortex of patients with temporal lobe epilepsy and mesial sclerosis, and in patients with cryptogenic frontal lobe epilepsy (Ferrer *et al*., 1994). These authors concluded that, because in these patients no morphological abnormalities were found in standard histopathological preparations and because of the lack of correlation between abnormal areas of staining and electrically abnormal foci recorded with electrocorticography, the existence of these patches might be explained simply as non-specific fluctuations in the immunocytochemical staining due to the release of calcium from intracellular calcium-binding proteins during depolarization of the cells. Later studies showed that the patches of decreased immunostaining for parvalbumin were coincident in adjacent sections with patches of decreased immunostaining for the GABA-synthesizing enzyme glutamic acid decarboxylase (Marco *et al*., 1996). Furthermore, using correlative light and electron microscopic methods to examine quantitatively the synaptic density in the neuropil of some of the regions showing focal decreases, these zones were also found to correspond to fine disorganizations in synaptic circuits which consist of increases and decreases of asymmetrical (presumptive excitatory) and symmetrical (presumptive inhibitory) synapses, compared with the adjacent normal cortex (DeFelipe *et al*., 1993; Marco and DeFelipe, 1997). We suggest that these changes might be due to a focal proliferation of excitatory axon terminals (probably originating from the local axonal arborizations of neighbouring pyramidal cells) and loss of inhibitory terminals, which may lead to hyperexcitatory synaptic circuits.

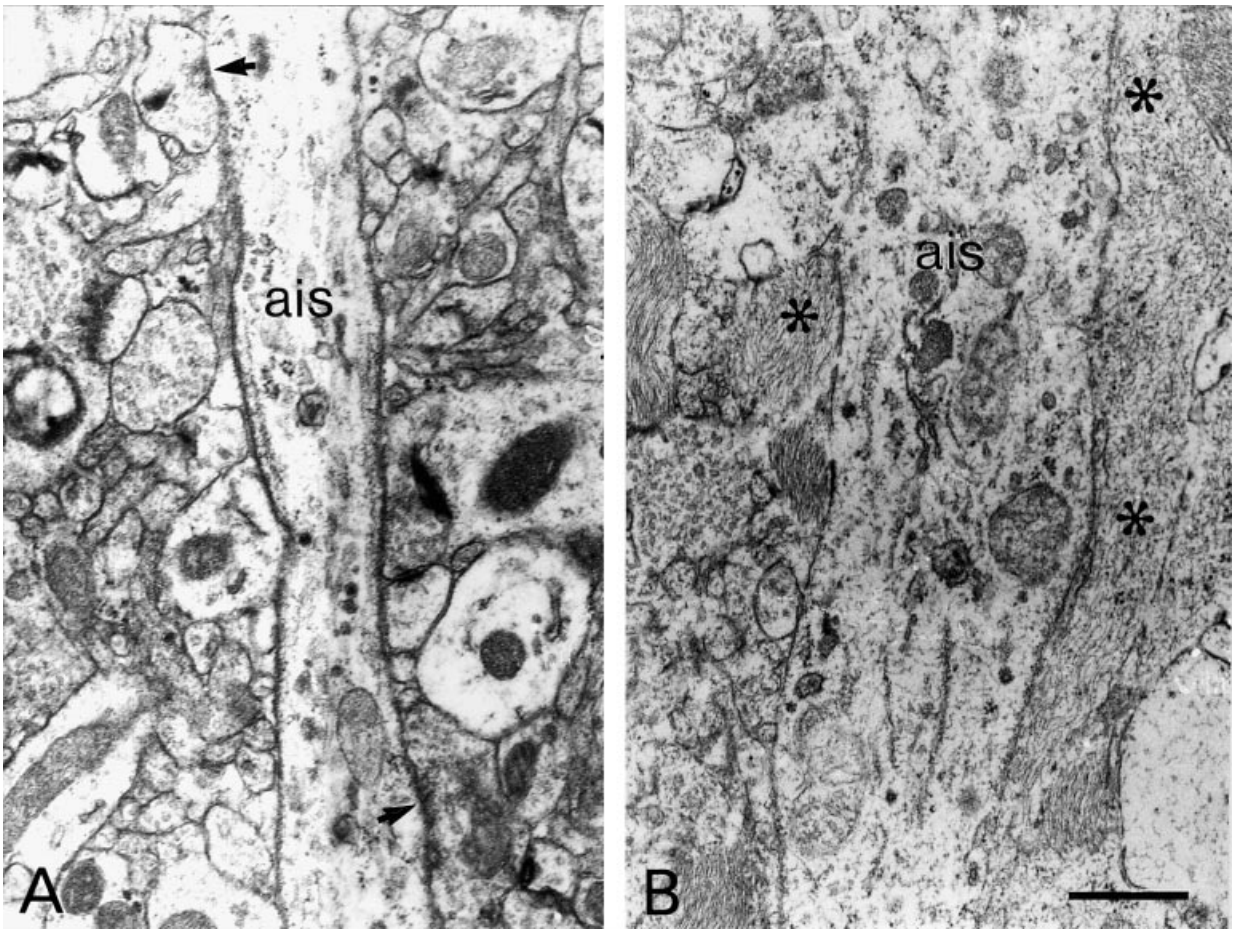


Fig. 7 Electron micrographs of pyramidal cell axon initial segments (ais) from the normal temporal cortex (**A**) and from a peritumoural (anaplastic astrocytoma) neocortical region showing no chandelier cell axon terminals stained for parvalbumin (**B**). In the normal cortex, several axoaxonic synapses (arrows) are observed, whereas in the peritumoural region the surface of the axon initial segment is apposed to glial processes (asterisks) and no axo-axonic synapses are found, which indicates an actual loss of chandelier terminals. Scale bar: 0.5 μm. (From Marco *et al*., 1997.)

The pathophysiological significance of intraoperative electrocorticography recordings is unclear because of the lack of a good correlation between seizure outcome after surgery and the localization of regions with maximal spike incidence or amplitude (Engel *et al*., 1975; Cascino *et al*., 1995; Schwartz *et al*., 1997). As pointed out by Alarcon *et al*. (Alarcon *et al*., 1997), among others, this is probably due to the complexity of electrocorticography and the difficulty in distinguishing between primary and propagated spikes simply by visual inspection. Primary spikes would be associated with structural lesions (i.e. epileptogenic regions), whereas regions with propagated spikes would be normal (see Alarcon *et al*., 1997, and references therein; see also Marco and DeFelipe, 1997). Further studies are necessary to investigate the relationship between patches of decreased immunostaining for parvalbumin and electrocorticography, using more sophisticated methods (such as computerized analysis of spike propagation proposed, for example, by Alarcon and colleagues) to identify epileptogenic zones. Nevertheless, as occurs with many pathologies associated with brain disease, a particular alteration is often difficult to ascertain as the cause or the consequence of the disease. For example, hippocampal sclerosis, which is often considered to be a primary lesion associated with epilepsy, has been shown to be induced after repeated kindled seizures in the rat (Cavazos *et al*., 1994). Also, seizures may not be present in patients with severe damage of the hippocampal formation and amygdala (Corsellis, 1957; Haymaker *et al*., 1958; Gloor, 1991). Thus, it is also possible that in some patients epileptic activity originating primarily in the neocortex or in the cortex of the parahippocampal gyrus induces neuronal loss in the hippocampus.

In conclusion, regions of neocortex showing decreases in parvalbumin immunoreactivity and altered synaptic circuits may be particularly relevant to epileptogenesis, but we do not know whether these changes are consequences of epileptic seizures or represent key components of a primary process that initiates the development of seizures. I propose a hypothesis below which supports the idea that the non-specific loss of chandelier cells may lead to seizures.

A chandelier cell hypothesis: variability in numbers of interneurons and non-specific loss of chandelier cells as a risk for the development of epilepsy

Since a primary pathology found in an epileptic patient may also be present in patients who are not epileptic (e.g. Meldrum and Bruton, 1992), what alterations are necessary in the neuronal circuitry to induce epilepsy, and why does this happen in some patients but in others it does not? It has been argued that a genetic component appears to be an important factor in the development of both idiopathic and symptomatic epilepsy (Falconer, 1971*b*; Treiman and Treiman, 1993; Cendes *et al*., 1998). It is possible that there is a normal, genetically determined variability in the number of interneurons which might predispose some individuals to develop epilepsy more than others in the face of a lesion or other precipitating factor that leads to neuronal loss. Evidence for variability comes from several quarters. First, the well-known differences in brain size suggest a considerable variability in the constitutive elements of the brain and their connections (e.g. see Purves *et al*., 1996; Peters *et al*., 1998). Secondly, it is the experience of many researchers working with human brain tissue (including ourselves) that there are great interindividual differences in the number of cortical interneurons labelled for GABA or calcium-binding proteins. In the monkey cerebral cortex, evidence for these differences is seen in counts of GABA neurons, which may vary considerably between one monkey and another (see Hendry *et al*., 1987). At the electron microscope level, significant interindividual differences have been demonstrated in the number of inhibitory synapses in the cat visual cortex (Beaulieu and Colonnier, 1985). We have observed variations of up to 30% in the density of symmetrical synapses in the human temporal neocortex (unpublished work). Although no data for variability in the number of chandelier cells is available from normal cortical areas, I propose (Fig. 8) that there may be individuals whose brains have relatively low numbers of GABA neurons and axon terminals ('$GABA_L$ type' brains), while the brains of other individuals have high numbers ('$GABA_H$ type' brains), and that the same factor inducing a loss of interneurons may produce epilepsy in one individual but not in the other.

This hypothesis is based on the fact that the GABAergic inputs on the dendrites and somata of pyramidal cells originate from many and diverse types of interneurons (for recent publications, see Kawaguchi and Kubota, 1997, 1998; Tamás *et al*., 1997; Thomson and Deuchars, 1997; Somogyi *et al*., 1998), whereas at the level of the axon initial segment, virtually all synapses arise from one or a few chandelier cells (Fairén and Valverde, 1980; Peters *et al*., 1982; Freund *et al*., 1983; Somogyi *et al*., 1983*c*; reviewed in Somogyi *et al*., 1982; DeFelipe and Fariñas, 1992). The number of GABAergic synapses which a pyramidal cell may receive has been estimated to be in the order of several hundreds (e.g. see DeFelipe, 1997). However, cells with axons ending on dendrites or somata form relatively few synapses with a given pyramidal cell (in the order of tens or less; e.g. Peters and Fairén, 1978; Somogyi *et al*., 1983*a*; Kisvárday *et al*., 1985; Tamás *et al*., 1997, 1998). Consequently, the loss of a few such cells might have little impact on the inhibitory control of the pyramidal cell, whereas if chandelier cells were affected, it would be crucial. The loss of chandelier cells could be selective or non-selective as a response to a given precipitating factor. However, as far as I know, there is no lesion or precipitating factor which is epileptogenic *per se* since, as pointed out above, the primary pathology or precipitating factor may also be present in patients who are

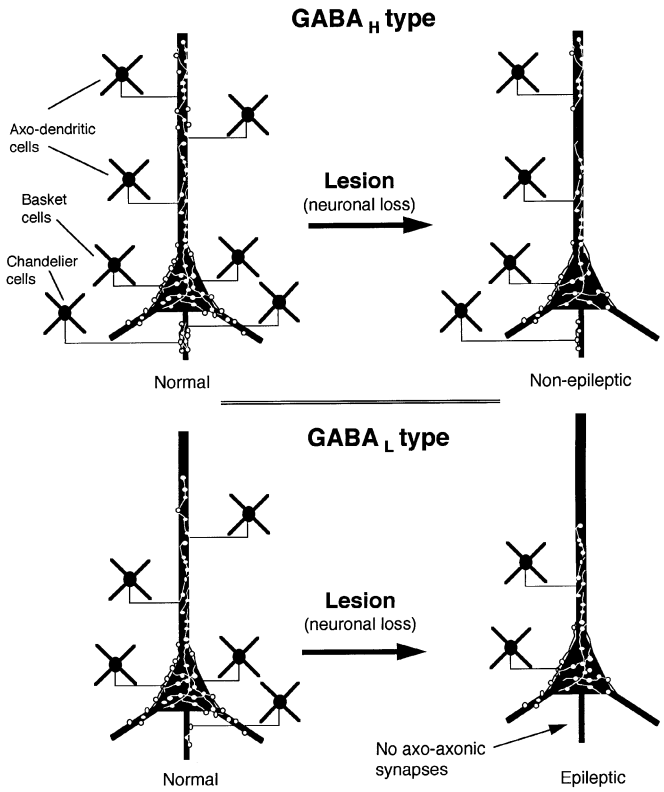


Fig. 8 Schematic diagram explaining the chandelier cell hypothesis for the development of epilepsy. This hypothesis is based on two facts. First, that the source of GABAergic inputs on the dendrites and somata of pyramidal cells (white dots) originates from numerous and various types of axo-dendritic cells and basket cells (or neurons that form synapses preferentially with dendrites or somata, respectively), whereas the main source of these synapses on the axon initial segment is from virtually only one or a few chandelier cells. Therefore, a loss of a few axo-dendritic and/or basket cells might have little impact on the inhibitory control of the pyramidal cell, whereas if chandelier cells are affected, the inhibitory control of the pyramidal cell would be strongly affected. Secondly, that there is a normal variability in the number of GABAergic interneurons, so that there may be individuals whose brains have relatively low numbers of GABA neurons and axon terminals ('$GABA_L$ type' brains), while other individuals have high numbers ('$GABA_H$ type' brains), and that the same factor inducing a loss of interneurons may produce epilepsy in one individual while not in the other, even though the same number and types of interneurons are lost, depending on the non-specific loss of chandelier cells.

not epileptic. Thus, if a given precipitating factor did produce a selective loss of chandelier cells, then many more epileptic patients should be expected.

Finally, since chandelier cells are also components of neuronal circuits in the entorhinal cortex, subiculum, hippocampus and fascia dentata (see section 'What is a chandelier cell?'), this hypothesis may also be valid in cases which present alterations only in these cortical regions. For example, Freund and Buzsáki found a selective loss of chandelier cell axons in rat hippocampal transplants which showed epileptiform activity (Freund and Buzsáki, 1988). Work in progress in our laboratory suggests that there is a decrease in chandelier cells in the apparently histologically normal subiculum and/or entorhinal cortex in epileptic patients who show a severe neuronal loss in the CA1 field. This may be particularly relevant in epileptic patients in whom seizure onset is restricted to mesial temporal structures and who present hippocampal sclerosis. As mentioned above, the incomplete removal of the parahippocampal gyrus, and particularly of the subiculum, in these patients resulted in a less satisfactory outcome (Siegel *et al*., 1990). Therefore, it has been hypothesized that the histologically normal parahippocampal gyrus may serve as a hyperexcitable fringe area which would amplify the relatively few excitatory outputs from the sclerotic Ammon's horn (Babb and Brown, 1986; Wieser, 1986; Siegel *et al*., 1990; Wieser *et al*., 1993). Our findings support this amplifier hypothesis, because the loss of these interneurons would contribute to the hyperexcitability of subicular pyramidal cells. In conclusion, it seems that the loss of chandelier cells might be non-specific and that when this occurs epilepsy may develop. Therefore, these cells might represent a key component in the aetiology of human epilepsy.

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