

Neuropathology of epilepsy

MARIA THOM

Department of Clinical and Experimental Epilepsy, Institute of Neurology, University College London

The effects of seizures on the brain are complex and have to be disentangled from the consequences of any primary neurological disease process that has led to increased seizure susceptibility. Although there is strong evidence to support detrimental effects of seizures on brain function and structure in many cases, this is by no means universal and still widely contested^{1,2}. Furthermore, any injurious effects of seizures should be evaluated not only by structural changes, such as neuronal loss and gliosis, but alterations at the cellular, synaptic and molecular level, some of which may be reversible and others permanent. Prolonged seizures may result in neuronal death by apoptotic (programmed or 'active') or necrotic pathways. Based on classical morphological definitions, cell necrosis would appear the dominant mechanism³, the exception being the dentate gyrus granule cells, including newly generated neurones in this region, that more readily show apoptosis⁴.

Post mortem examinations in epilepsy

In the examination of a brain from a patient with epilepsy, the neuropathologist addresses three questions in relation to this disease: (i) can a cause for the epilepsy be identified; (ii) are secondary changes as a result of seizures present; and (iii) the potential contribution of epilepsy to the cause of death. For the investigation of the former, tissue sampling is influenced by any macroscopic abnormality identified or from localising clinical, electrophysiological and/or neuroimaging data. For secondary changes, regions of brain most vulnerable include the hippocampus, neocortex, thalamus, amygdala and cerebellum.

Epilepsy-related deaths

Post mortems carried out in patients with epilepsy include both Coroners' examinations (in particular in sudden and accidental deaths) and hospital post mortem requested by the clinician with consent of the family. In many cases the bereaved family are happy to donate brain tissue for research programmes to further advance studies in epilepsy.

Status epilepticus

Neuropathological findings in fatal cases of status epilepticus (SE) include acute and extensive loss of hippocampal pyramidal neurones⁵ which may be unilateral⁶ or bilateral⁷. Human post mortem studies in patients with recent SE (without a prior history of epilepsy, evidence of cerebral hypoxia, or other systemic complications), have demonstrated neuronal loss in CA1, CA3 and the hilus (the dentate granule cells may be spared⁷), amygdala (corticomedial and basolateral nuclei), neocortex (mid cortical layers), the entorhinal cortex, Purkinje cell layer of the cerebellum⁸, mamillary bodies⁹, the dorsal medial nuclei of the thalamus¹⁰ and basal ganglia⁷. The neuronal damage may be predominantly unilateral in some cases¹⁰ and with histories of prolonged hemi-convulsions cerebral hemiatrophy can eventually occur with



Extensive contusion in fronto-basal region in patient with epilepsy and polymicrogyria (arrowed)

striking unilateral laminar necrosis of the second to fourth cortical layers¹¹. Quantitative neuropathological studies in a subgroup of patients with long histories of generalised seizures, including frequent episodes of SE, also show hippocampal¹² and neocortical⁸ neuronal loss is not an inevitable consequence of SE.

Traumatic brain injury

Traumatic lesions are a common finding in patients with epilepsy. Patients with established epilepsy have a higher incidence of minor and severe cerebral injuries, including cerebral haemorrhage and contusions; the risk is related to seizure frequency, type and control¹³. Post mortem examinations of patients with longstanding idiopathic seizures or refractory epilepsy may not infrequently reveal sequelae of previous head injuries including old cystic cortical contusions, particularly in the fronto-temporal regions. This may increase vulnerability to age-accelerated neurofibrillary tangle pathology.

Sudden death in epilepsy

Neuropathology. Post mortem examination is mandatory in suspected sudden unexpected death in epilepsy (SUDEP) and is essentially a process of exclusion of an anatomical or structural cause of death. Post mortem is carried out under the jurisdiction of the Coroner. The examination of the brain in SUDEP cases may show mild swelling or 'fullness' of the convexities reflected in high-average brain weights but, by definition, significant swelling, shift or herniation is absent¹⁴. It is a perhaps a common misconception that the brain in SUDEP cases is normal in the vast majority of cases. Analysis from the larger SUDEP series report macroscopic abnormalities in half to two-thirds of cases^{15,16}. More frequently reported macroscopic abnormalities include old cerebral traumatic lesions (contusions, gliosis, previous craniotomy sites), hippocampal or cortical atrophy, cerebellar atrophy, haemangiomas, low-grade tumours and cortical malformations¹⁵. There is no accurate data regarding the relative risk or association of any of these specific pathological lesions for SUDEP. Some lesions, including old injuries and cortical neuronal damage, however, may give an indirect measure of the clinical severity of the epilepsy. Histopathological examination is required in SUDEP cases for the confirmation of any type of macroscopic lesion identified but also in cases which appear normal to exclude significant pathology.

It is not possible or necessary for a neuropathologist to perform all the autopsies on patients with epilepsy. Ideally though, a specialist neuropathologist should be involved in the interpretation of the histological findings. The Royal College of Pathologists' guidelines on autopsy practice in epilepsy recommends that a case should be made to the Coroner and relatives for retention of the whole brain for fixation. This allows optimal examination following 2–3 weeks' fixation. If this is not permissible the next best process is to fix coronal slices of the brain (taken 1.5 cm thick just in front of the midbrain and just behind the midbrain) for a short period (2–3 days) followed by photography and histopathology sampling. If even this is not permissible then small tissue samples must be selected and trimmed for histopathological analysis and the brain immediately returned to the body at time of autopsy. It has been shown in SUDEP series that if the brain is cut and examined in fresh rather than a

fixed state then there is less likelihood of identifying any relevant pathology¹⁶. The coroner and family should be made aware of this limitation when retention of the brain is denied.

Other organ pathology in SUDEP. There have been several studies addressing the presence of associated or significant cardiac pathology in SUDEP which may relate to the cause of death. Initial reports suggested increased heart weights and co-existing cardiac hypertrophy in some patients with SUDEP¹⁷. In more recent studies however, no difference in heart mass compared to non-SUDEP controls was noted when corrected for body mass^{18,19}. Extensive sampling of the myocardium in SUDEP in one study revealed frequent foci of reversible pathology (myocyte vacuolisation and interstitial oedema) in addition to irreversible pathological changes (contraction band necrosis, haemorrhage, fibrosis and hyper-eosinophilia of myocardial fibres) compared to control groups. Regions of myocardial fibrosis have been described around vessels or interdigitating between bundles of fibres¹⁹. In a further study, 13 blocks of myocardium were sampled from each of 23 SUDEP cases and a significant increase in deep and subendocardial fibrosis was shown in 40% of the SUDEP patients compared to controls²⁰. Cardiac fibrosis has not however been reported in all post mortem SUDEP series¹⁷. The current Royal College of Pathologists guidelines for autopsy practice in epilepsy deaths recommend that three blocks of left ventricle and one block of right ventricle are sampled to exclude vascular-ischaemic damage or other cause of cardiac death as myocarditis. This limited sampling may mean that smaller foci of cardiac fibrosis are missed and more generous sampling protocols of up to 10 blocks per case, as in the investigation of other sudden adult death cases²¹, may be a more cautious approach. Pulmonary oedema has been reported in 50–90% of SUDEP cases^{14,15,17}. Lung weights in SUDEP cases did not differ from non-SUDEP cases¹⁸ in one study. Toxicology screening is important in the investigation of SUDEP, as in other adult sudden death cases, in order to exclude a toxic cause of sudden death and for the monitoring of AED levels to assess compliance. This should include blood, urine, and gastric contents for AEDs, drugs of abuse and alcohol level estimations. Vitreous humour should be taken for biochemistry if diabetes or other metabolic disorder is considered. Hair testing may also prove useful to test for long-term drug compliance if indicated²². A recent study in 68 SUDEP autopsies confirmed ion channel gene mutations observed in LQTS in 13%²³.

SUDEP: recognition, likely mechanisms and future directions. There are no neuropathological diagnostic features of SUDEP but establishing this category allows such cases, which fit a pattern, to be grouped together and identified. The ‘U’ in SUDEP could equally stand for ‘unexplained’; as yet, as commented in a recent Lancet review, we are still at the stage of ‘hypothesis generation’²⁴. Epidemiological studies and current research to date support the notion that SUDEP is an ictal event and that cardiac, pulmonary or autonomic dysfunction concurrent with a seizure are the main mechanistic contenders. SUDEP is also likely to be multifactorial, with different causal mechanisms contributing in each case.

Recognition of SUDEP had been one of the main obstacles prior to the 2002 National Sentinel Audit in the UK. Guidelines for best practice in epilepsy deaths were subsequently issued in 2005 by the Royal College of Pathologists. A national confidential enquiry (NCEPOD) in 2006 into Coroners’ autopsies, however, continued to single out post mortem examinations in epilepsy deaths as an area of specific concern, including the brain examination. This would suggest that the practice is still not perfect. If we are going to make progress in understanding what causes SUDEP, which is one step towards its prevention, a ‘global action’ is required²⁵ with teamwork between multidisciplinary professionals, including neuropathologists.

Surgical neuropathology and focal epilepsies

Epilepsy surgery has been established as a reliable treatment option in pharmaco-resistant focal epilepsies and with the advances in imaging technology, structural lesions are increasingly recognised in patients with chronic focal seizures. As the epileptogenic area often extends beyond the structural or visible lesion it requires careful electrophysiological evaluation, for example surface or invasive EEG recording. Thus, an interdisciplinary approach combining histopathological and molecular-biological studies of electrophysiologically well-characterised brain tissue is required to clarify the aetiology, biological behaviour, and functional impairment of epilepsy-associated structural lesions. Addressing molecular pathomechanisms may also unravel novel pharmacological targets in the human brain².



Surgical temporal lobectomy specimen received fresh in laboratory (and hippocampus below) allows correct orientation and sampling to optimise both diagnostic and research work.

Epilepsy surgery has been performed for over a century but in the last 20 to 30 years has taken on a more important role in management of refractory seizures, largely due to advances in neuroimaging and surgical methods. This trend is likely to continue based on the prevalence of patients living with ‘surgically remediable syndromes’ and because surgery has been shown to be generally successful and safe compared with continued trials of conventional drug treatments^{26,27}, with up to two-thirds of patients becoming seizure free.

In many centres surgical tissues are sent immediately, fresh to the laboratory to allow the opportunity for appropriate freezing, fixing and banking tissue samples for both diagnostic and research purposes²⁸.

The major surgical pathologies in patients with temporal lobe epilepsy (TLE) are divided into two types; hippocampal sclerosis in around 60–70% of cases²⁶ and mass or lesional pathologies (including dysplasias and tumours) in about 30–40%. In a proportion of patients the hippocampus will be removed together with a lesional pathology (either temporal or extra-temporal). In these cases, variable degrees of hippocampal atrophy may be present, sometimes referred to as ‘secondary’ hippocampal sclerosis or *dual* pathology; these account for between 6–16% of cases in different epilepsy series.

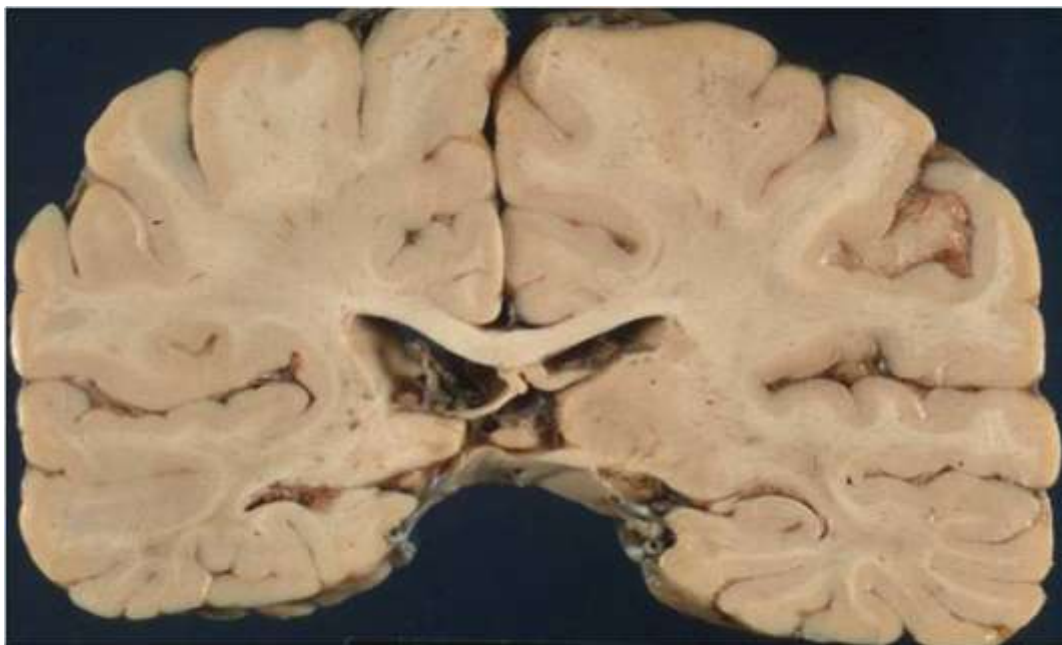
In a variable proportion of patients (around 7% in one large series²⁹), no pathology is identified in the resected temporal lobe (also referred to as ‘cryptogenic’ or ‘paradoxical’ TLE)³⁰.

<i>Diagnosis</i>	<i>subtype</i>	<i>number</i>	<i>Age at surgery(yrs)</i>
Hippocampal sclerosis		1591	34.6
Dual pathology		218	24.8
Long-term epilepsy associated tumours	All	1236	28.4
	Ganglioglioma	570	25.4
	DNT	189	20.3
Dysplasia	All	577	18.5
	FCD I	66	11.5
	FCDII	271	18.4
Scars		239	25.2
Encephalitis		73	22.0
No lesion		307	29.2
Vascular		271	36.4

Adapted from Blumcke². All data were obtained from the German Neuropathological Reference Center for Epilepsy Surgery with 4512 cases in total. FCD, focal cortical dysplasia; DNT, dysembryoplastic neuroepithelial tumour

Hippocampal sclerosis

Hippocampal sclerosis (also referred to as mesial temporal sclerosis, MTS, or Ammon's horn sclerosis, AHS) describes atrophy of the hippocampus with a stereotypical pattern of neuronal loss and gliosis. This manifests as a reduction in the volume of this structure as seen on neuroimaging or macroscopic examination. Hippocampal sclerosis is strongly associated with the clinical syndrome of mesial temporal lobe epilepsy (MTLE). The neuropathological features have been recognised for over a century (for historical review see Thom³¹) but its cause, and in turn how it causes epilepsy, is still the focus of extensive ongoing research, utilising both human tissue and experimental models.

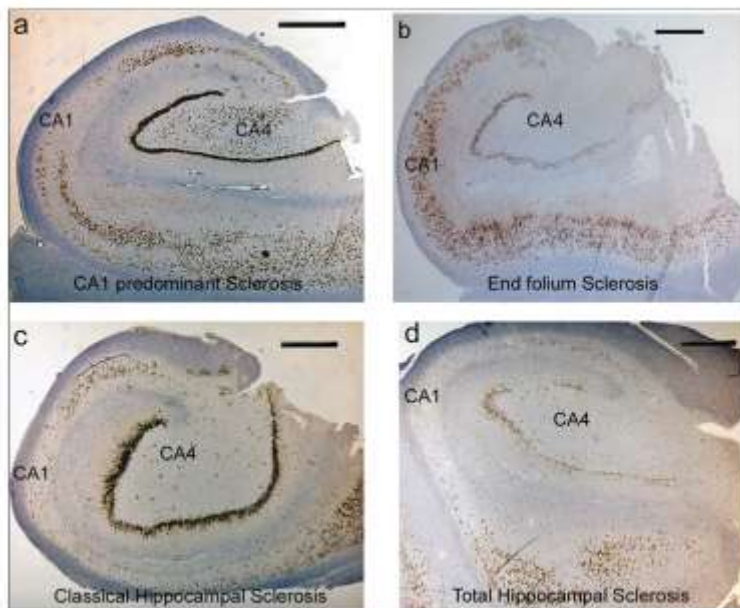


Unilateral hippocampal sclerosis seen at post mortem through a coronal slice at the level of the pulvinar of the thalamus.

Patterns of sclerosis

The pathological diagnosis of hippocampal sclerosis is based on the identification of pyramidal neuronal loss and gliosis involving mainly CA1, CA4 and CA3 subfields, as well as other hilar neurones (including NPY and mossy cells). This distinctive pattern of neuronal loss is apparent on qualitative histological examination and may even be evident on visual examination of the surgically resected tissue and is also referred to as ‘classical’ hippocampal sclerosis. Quantitative techniques can reveal more subtle, widespread neuronal loss in other subfields, for example the dentate granule cell layer. CA2 sector is more resistant to neuronal loss and often the pyramidal cells in this region appear better preserved, as do those of the subiculum. The granule cell layer may appear normal but in 40–50% of cases shows dispersion or more loose packing of the neurones. Widespread depletion of granule cell neurones is present in approximately 5% of surgical cases and when associated with widespread pyramidal cell loss (including CA2) is referred to as ‘total’ hippocampal sclerosis. NeuN or MAP2 immunohistochemistry can be a useful aid to identify the vestiges of the granule cell layer in these cases. End folium sclerosis describes a pattern of neuronal damage confined to the hilar and CA4 pyramidal cells and is seen in approximately 3% of patients in surgical series. There is no single explanation for the regional selectivity of pyramidal cell loss between subfields; excitatory pathways, altered inhibitory input, and the effectiveness and variability of endogenous neuro-protective mechanisms are likely to be involved.

Several semi-quantitative schemes have been used for grading the severity, as well as patterns of neuronal loss, mainly for correlation with clinical parameters and neuroimaging appearances. Examples are the schemes proposed by Wyler³² and more recently by Blumcke et al³³. This last classification system, using a quantitative analysis scheme, categorises patterns of hippocampal sclerosis as MTS type 1 (a classical pattern of neuronal loss in CA1 and CA4 predominantly), MTS type 2 (neuronal loss predominantly in CA1 sector) and MTS type 3 (end folium sclerosis).



Main patterns of hippocampal sclerosis described in surgical and post mortem tissues. All sections through body of hippocampus and stained with NeuN antibody.

There is some evidence to suggest that although the atypical patterns (non-classical) of hippocampal sclerosis are less common, they are associated with worse outcome in terms of seizure freedom following surgical resection. This was also observed in a recent series from Queen Square, however whereas patients with CA1p predominant sclerosis gained less benefit from surgery, patients with end folium sclerosis fared better in this series³⁴. Below is a table which compares the findings relating to patterns of neuronal loss and outcome in published series³⁴.

Table 3. Comparisons of main findings in previously reported surgical studies comparing atypical patterns of hippocampal sclerosis and epilepsy history

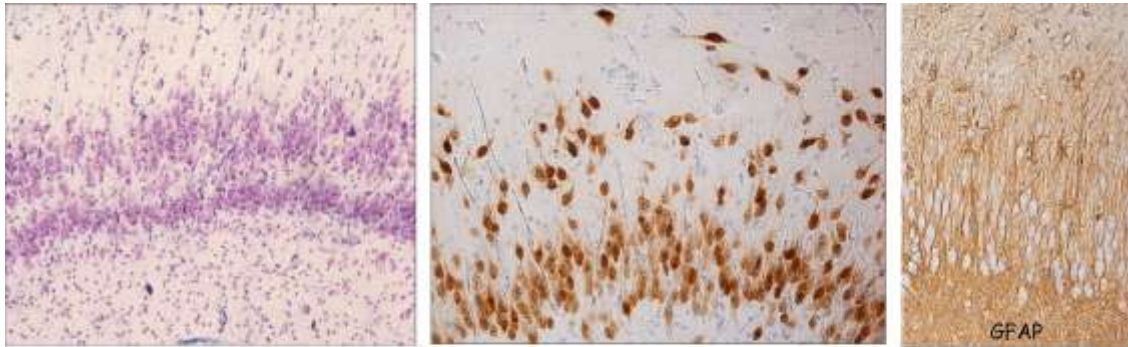
Pattern of HS	Sagar and Oxbury (1987)	Bruton et al. (1988)	Davies et al. (1996)	Van Paesschen et al. (1997)	de Lanerolle et al. (2003)	Blumcke et al. (2007)	Present series
CHS	CHS	CHS	CHS	CHS	CHS	CHS/MTS type 1	CHS
No. of cases	12	61	59	52	72	33	60
Age onset	9.1 years	5.19 years	7.4 years	7 years	4.4 years	10.3 years ^b	9.3 years
IPI (FS)	No data	48% (FS)	No data	62% (FS)	No data	76% (FS)	50% (FS)
Outcome	No data	37% (SF) ^a	68% (SF)	81% (SF)	84.5% (SF)	72% (SF)	69% (SF)
EFS	EFS+CAIP ^c	EFS	EFS+CAIP ^c	EFS	EFS	EFS/MTS type 3	EFS
No. of cases	10	4	8	4	No cases	7	5
Age onset	13.2 years	18.5 years	12.4 years	15.5 years		14 years	18.2 years
IPI type (FS)	No data	0% (FS)	No data	0%		0%	80% (FS)
Outcome	No data	50% (SF)	66.7% (SF)	25% (SF)		28% (SF)	100% (SF)
CAIP		CAIP		CAIP	CAIP	CAIP/MTS type 2	CAIP
No. of cases		No cases		No cases		9	9
Age onset					7.9 years	15 years ^b	5.9 years
IPI type (FS)					No data	75% (FS)	62.5% (FS)
Outcome					77.8% (SF)	66.7% (SF)	33% (SF)
No HS	No HS	No HS	No HS	No HS	No HS	No HS	No HS
No. of cases	10	38	31	No cases	18	34	17 cases
Age onset	11 years	15.37 years	19.9 years		9.4 years	18.4 years ^b	12.4 years
IPI type (FS)	No data	2.6% (FS)	No data		No data	0% (FS)	35% (FS)
Outcome	No data	20% (SF) ^a	42.3% (SF)		44% (SF)	58.6% (SF)	44%
Significance reported between groups	p < 0.02 (age onset)	No statistics reported between HS groups Follow-up period, 8 years (mean).	p < 0.001 (age onset) p < 0.003 (IPI type, FS) p < 0.01 (outcome)	p < 0.02 (age onset) p < 0.03 (IPI type, FS) p < 0.04 (outcome) Follow-up period 1 year (Engel)	p < 0.05 (age onset) Follow-up period 1 year (Engel)	p < 0.01 (age onset) p < 0.001 (IPI type, FS) p < 0.04 (outcome) Follow-up period 1 year (Engel)	p < 0.01 (age onset) p < 0.05 (outcome) Follow-up period 2 years (ILAE)

Age onset, age of onset of habitual seizures; IPI, initial precipitating injury; FS, febrile seizures; SF, seizure free; Outcome, seizure control following surgery (using either Engel, ILAE grading (Wieser et al., 2001)).
^aSeizure pattern greatly improved following surgery; MTS, mesial temporal sclerosis.
^bHabitual seizure onset age calculated by adding age at initial event and latency period.
^cIn these series CAIP and EFS HS grouped together as one category/grade.

Associated findings with hippocampal sclerosis

(i) *Dispersion of granule cells* into the molecular layer, associated with hippocampal sclerosis, was first described by Houser³⁵. This phenomenon appears peculiar to seizure-induced hippocampal damage and is encountered in between 40–50% of HS cases in surgical series³⁶⁻³⁹. In the presence of dispersion, granule cells often appear enlarged and more fusiform in shape, with increased cytoplasm and neuropil separating neurones. In some cases ectopic neurones within the molecular layer have their long axis orientated more horizontally^{37,40}. The border of the granule cell layer with the molecular layer, as a consequence of this neo-migration, becomes more ill-defined. In some cases distinct clusters of granule cells are seen in the molecular layer and, in a smaller number (about 10% of surgical cases), a bi-laminar granule cell layer is noted (see Figure over, top). The extent and pattern of dispersion may vary both within and between cases and may alternate with regions of granule cell depletion. There is no precise definition for granule cell dispersion (GCD); a granule cell layer thicker than 10 cells³⁸ or 120 μm has been proposed. In many cases the thickness may in fact reach 200 μm or greater⁴¹, compared to mean control widths of around 100 μm . A recent paper proposes a subclassification system for types of dispersion⁴², although no correlation with outcome following surgery has been demonstrated in any study^{34,42}.

The functional significance as well as the cause for granule cell dispersion is therefore unknown. Dispersion of granule cells has also been demonstrated in experimental models of TLE. For example, granule cell dispersion is observed in the kainate and domoate models, first appearing at about four days following seizures, increasing over eight weeks and persisting for at least six months⁴³. GCD is almost invariably associated with gliosis in the granule cell layer and it has been proposed that persistent radial glial processes guide this neo-migration of granule cells through the dentate gyrus⁴⁰.

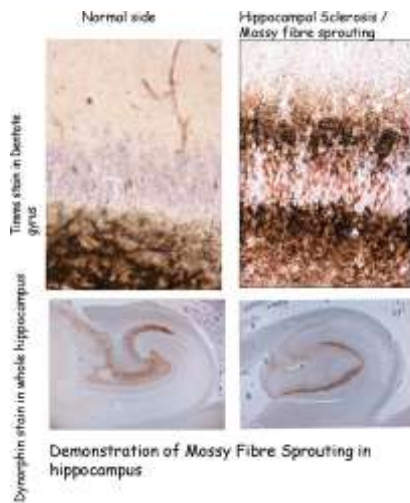


Patterns of Granule cell dispersion : A bilaminar pattern on the left, single dispersed neurones as seen with NeuN (centre) and the accompanying gliosis as seen with GFAP on right.

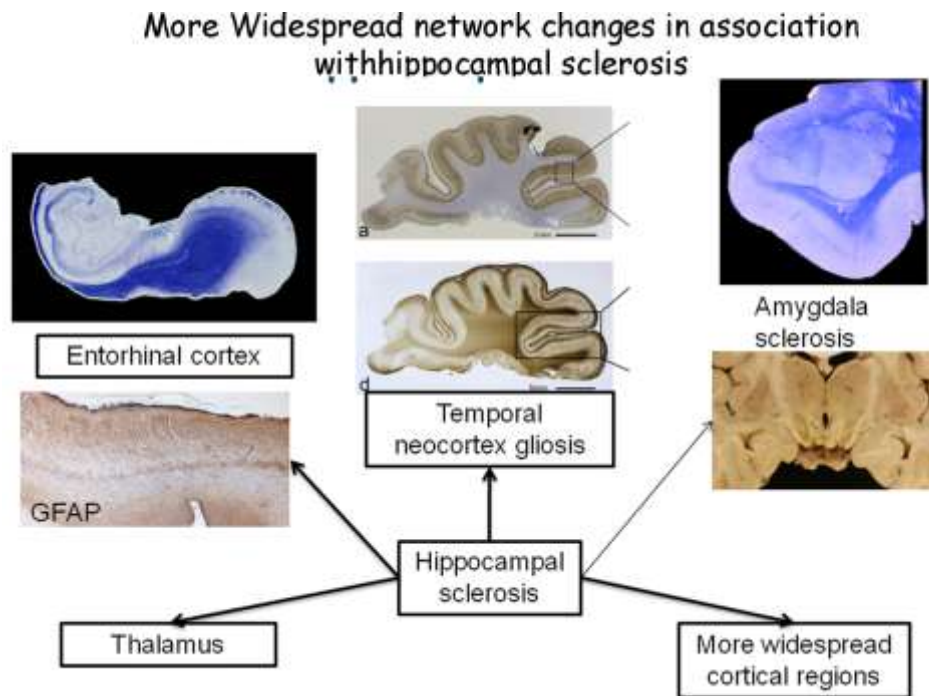
In the reeler mouse model, in addition to the widespread cortical abnormalities observed, dispersion of granule cells is present throughout the hilus and molecular layer⁴⁴ which is similar to observations in hippocampal sclerosis. Reelin protein is considered essential for the normal positioning of the granule cells, probably through the maintenance of a normal glial scaffold⁴⁵. There is accumulating data for a reelin deficiency acting in human GCD in the context of hippocampal sclerosis^{45,46}. The identification of neurogenesis in the adult hippocampus has also led to the notion that GCD in epilepsy is a consequence of excess neurogenesis stimulated by seizures but there is less evidence in support of this.

(ii) *Mossy fibre sprouting*. In animal models of MTLE (e.g. the kainate model), and in hippocampal sclerosis in humans, extensive recurrent projection of mossy fibre collaterals into the molecular layer occurs, a process more commonly known as mossy fibre spouting (MFS). The majority (over 90%) of these sprouted mossy fibres appear to make synaptic contact (excitatory asymmetric synapses)⁴⁷ with apical dendrites and spines of granule cells in the inner molecular layer, and a smaller proportion with inhibitory interneurons⁴⁷. This therefore creates a recurrent excitatory circuit, potentially a pro-epileptogenic ‘short-circuit’. However, recent post mortem studies in humans support the notion that MFS is more likely an epiphenomenon rather than directly provoking seizures⁴⁸. MFS is best visualised (in both experimental and human tissue) with Timm silver method. The mossy fibre boutons contain high levels of zinc, sequestered in synaptic vesicles and released together with glutamate. In the normal

hippocampus dense Timm staining in the hilus is seen, with no staining in the supragranular region. In the presence of extensive MFS, a dense confluent band of zinc-silver positive granules is seen in the inner molecular layer of the dentate gyrus. The Timm granules correspond to mossy fibre terminals on ultrastructural examination⁴⁹ and several granules may be present in a single mossy fibre synaptic terminal. In many cases the sprouted fibres in the inner molecular layer form a well-defined boundary with the outer molecular layer. MFS can also be demonstrated with immunohistochemistry for dynorphin A, an opioid neuropeptide that is normally present in the granule cells and in the terminal fields of the mossy fibres.



(iii) *Widespread changes in association with hippocampal sclerosis.* There is accumulating data in TLE that the anatomical abnormalities may extend far beyond the hippocampus. These additional pathologies may be relevant to poorer response of seizures to surgical treatments, as well as additional clinical features, including cognitive dysfunction.



Amygdala. Neuropathology studies have reported gliosis and neuronal loss in the lateral nucleus in resections of amygdala from patients with temporal lobe epilepsy, in particular the ventro-medial aspects are more severely affected⁵⁰. In addition, the basal nuclei, particularly the parvocellular division, may be involved⁵¹. In cases with severe neuronal loss and gliosis the term ‘amygdala sclerosis’ may be applied but there is no strict definition for the extent and severity of neuronal loss for this diagnosis³⁸. As a result, reports on the incidence of amygdala sclerosis vary between institutions from 35–76%⁵⁰. Greater amygdala neuronal loss has been shown in patients with hippocampal sclerosis although amygdala sclerosis has also been reported in isolation³⁸.

Entorhinal cortex (EC) in hippocampal sclerosis. There is also evidence that characteristic pathological changes are present in the entorhinal cortex in patients with hippocampal sclerosis and that this region may have importance in the initiation of mesial temporal lobe seizures or development of hippocampal sclerosis. The EC at the junction between hippocampus and neocortex acts as a conduit for incoming afferent information and reciprocal efferent signals. It also contributes to local signal processing and modulation and intracortical networks between the deep and superficial cortical regions have been shown. The EC has reciprocal connections with the hippocampus. Neurons from superficial layers (mainly layers II and III) send glutamatergic afferents, via the perforant pathway, to the dentate granule cells and CA1 neurons; subicular and CA1 pyramidal neurons have feedback connections to the deeper layers of the entorhinal cortex. Neuroimaging studies have reported volume reduction of parahippocampal gyral structures in TLE, mainly ipsilateral to the seizures⁵² and abnormal epileptiform activity has been recorded in the EC region⁵³, which may sustain seizures. In pathological studies of animal models of TLE, and post-status, selective vulnerability of layer

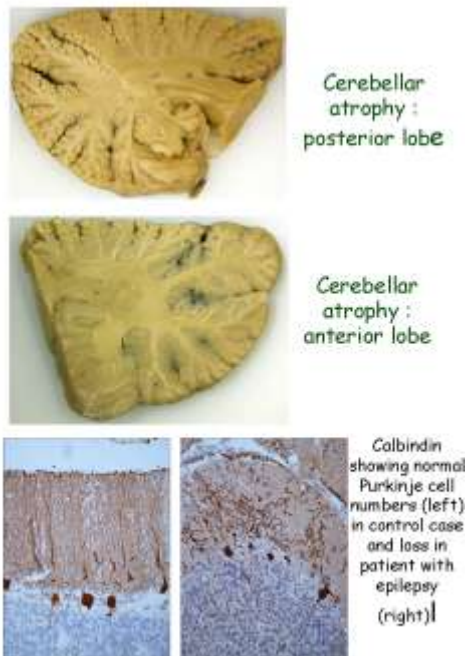
III neurones was shown^{54,55}. Recent quantitative studies of this region in patients with hippocampal sclerosis undergoing surgery have suggested more subtle and variable patterns of gliosis and neuronal loss with destruction of an entire lamina being relatively uncommon^{50,56}. In addition, there is evidence that GABAergic interneurons are relatively spared in this region. Ultimately, as the entorhinal cortex has a complex cytoarchitecture which defines its many subfields, often not completely represented in surgical specimens, significant regional pathology may remain unidentified. Human post mortem tissue may be better suited to address the question of the anatomical extent of any EC damaged associated with chronic hippocampal sclerosis.

Neocortex. In the neocortex, loss of neurones is most apparent in mid-cortical layers with associated gliosis. Subcortical white matter gliosis, and atrophy, Chaslin's superficial cortical gliosis and increased deposits of corpora amylacea may also be prominent. Mild, focal leptomeningeal chronic inflammatory infiltrates may follow a seizure. When extensive in the temporal lobe the pattern of temporal lobe sclerosis may be accompanied by reorganisational dysplasia, now termed FCD type III (see section below for dysplasia). (For review of temporal lobe sclerosis see Thom et al⁵⁷). Quantitative post mortem studies of patients with hippocampal sclerosis also support more widespread neocortical pathology⁵⁸.

Thalamus. Volume reduction of the thalamus has been observed with MRI studies⁵⁹, as well as in post mortem studies of epilepsy⁶⁰ and experimental studies⁶¹, and is more closely linked with hippocampal and amygdala atrophy and TLE. Neuronal loss and gliosis may be widespread or appear to involve specific nuclei such as the anterior and dorsomedial nuclei. Thalamic atrophy is more often unilateral and associated atrophy of the fornix and mamillary bodies may also been seen. Proposed patho-mechanisms for injury include direct effects of seizures or transneuronal degeneration via connecting pathways.

Cerebellum. In neuropathological studies of patients with a history of epilepsy, macroscopic atrophy of the cerebellum has long been identified⁸, present in 25% of cases in one post mortem series⁶⁰. It is generally regarded that cerebellar atrophy is likely to be acquired during the course of the epilepsy rather than a predisposing factor for seizures. MRI volumetric studies, for example, have shown increased atrophy in established epilepsy compared to newly-diagnosed patients⁶². Cerebellar atrophy has been observed in association with both generalised and focal seizures and in studies of patients with TLE; 4–6% cerebellar volume reductions have been shown using MRI⁶³. Neuropathological findings at post mortem may disclose preferential symmetrical atrophy of the anterior lobes or the more common pattern involving the posterior lobes⁶⁴. In mild cases damage may be restricted to a folium and in severe cases more generalised atrophy is observed. Crossed cerebellar atrophy (cerebellar diaschisis) is also recognised in patients with contralateral destructive cerebral hemispheric lesions associated with seizures, including hemiatrophy. The prevalence of crossed atrophy has also been investigated with MRI⁶⁵ and milder degrees of a cerebellar asymmetry may be disclosed in quantitative neuropathological studies of patients with a unilateral seizure focus⁶⁴.

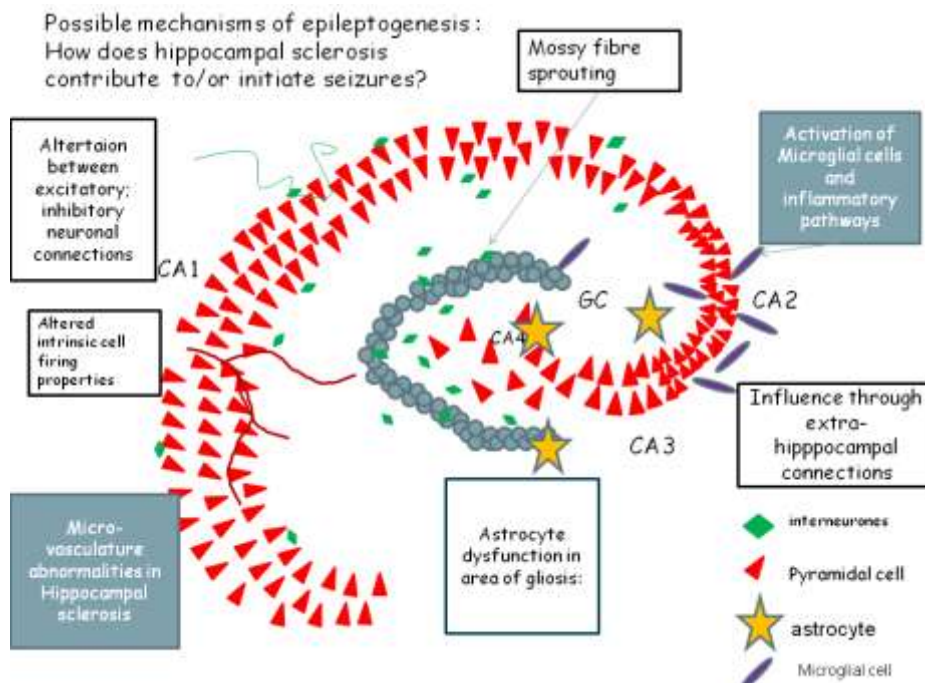
Regardless of the lobar distribution, the histological findings are Purkinje cell loss, Bergman gliosis in the cortex, relative preservation of basket cells, and granule cell damage. Occasional torpedo-like axonal swellings on Purkinje cells may be observed. The cause of the cerebellar atrophy has been attributed to seizure activity (in particular episodes of status epilepticus⁶⁵), antiepileptic drug (AED) toxicity (in particular phenytoin), hypoxic-ischaemic injury, trauma as a result of seizures⁶⁴ or trans-neuronal degeneration (particularly for the crossed cerebellar pattern).



In support of a seizure-induced pathogenesis, atrophy was documented before the introduction of anticonvulsant medications, and the observation of acute necrosis of the Purkinje cells following status (occasionally with a crossed pattern) implicates excitotoxicity. Phenytoin has been shown to cause cerebellar atrophy following acute and chronic administration⁶⁴, and experimental toxicity to Purkinje cells and granule cells has been shown^{66,67}. There is no consistent relationship between seizure frequency and duration and the degree of atrophy to argue for either process⁶²⁻⁶⁵. The observation that crossed atrophy may occur in the absence of seizures would favour trans-synaptic degeneration via cerebro-cerebellar pathways. In fact, all these mechanisms may be acting synergistically and may not easily be separated. Typically there are few clinical symptoms attributable to the atrophy but mild cognitive deficits may occur⁶⁸. Seizure control post temporal lobectomy was shown not to be influenced by the presence of cerebellar atrophy in one⁶⁹ but not another⁷⁰ study.

Causes of hippocampal sclerosis and mechanisms of epileptogenesis

Epileptogenesis refers to a process in which an initial brain-damaging insult triggers a cascade of molecular and cellular changes that eventually lead to the occurrence of spontaneous seizures⁷¹. Likely mechanisms operating in hippocampus are summarised in the diagram below.



Cortical malformations in surgical series

Focal cortical dysplasias

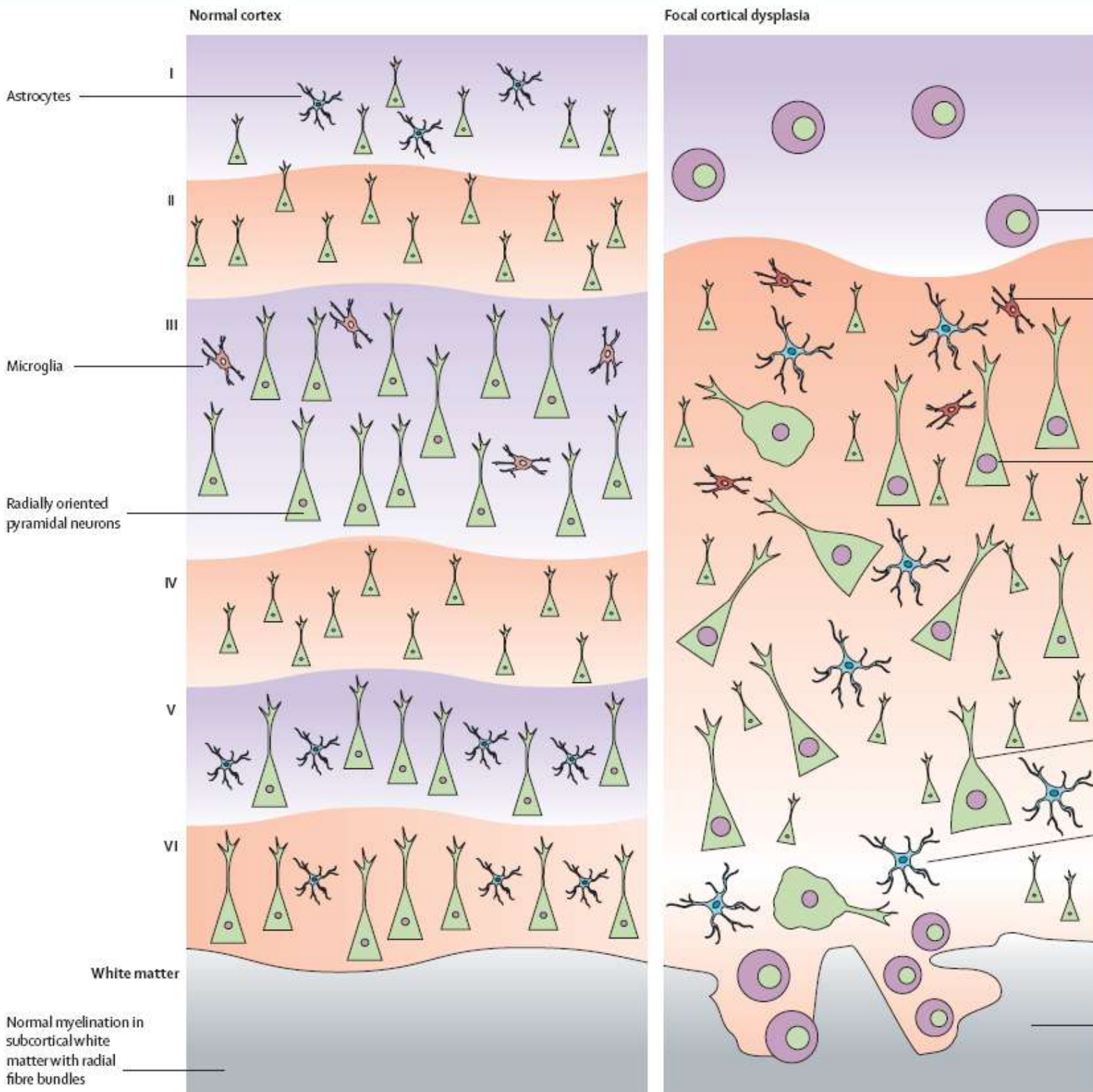
Cortical dysplasia refers to a subtype of malformations due to abnormal cortical development (MCD) where the abnormality is strictly or largely intracortical⁷². In some instances these lesions, if localised, are amenable to surgical resection. In the past a variety of different terms has been used for these abnormalities including ‘Taylor type dysplasia’ (after one of the initial descriptions of this lesion), ‘cortical dysgenesis’ and ‘microdysgenesis’. A reappraisal of the nomenclature was proposed by Palmini⁷², based largely on neuropathological features, in order to facilitate better correlation of neuroimaging, clinical-electrographic data and clinical characteristics of different lesion types between epilepsy centres. The Palmini system has two main groups: the focal cortical dysplasias (FCDs) and the mild MCDs. The FCDs were divided into two types: type I (cytoarchitectural abnormalities without balloon cells or dysmorphic neurones) and type II (cytoarchitectural abnormalities with abnormal neurones or balloon cells). These types are then further divided representing a spectrum of increasing severity of pathological dysplasia. A revised ILAE classification of cortical dysplasias was published in 2011 which includes an additional tier of dysplasias (type III) seen in association with a second pathology (including hippocampal sclerosis, tumours, scars and vascular lesions)⁷³. The rationale is to separate isolated FCD (type I and II) from these associated dysplasias which are likely to have a different aetiology (linked with the primary pathology), may represent acquired changes in the developed cortex rather than developmental lesions and have different prognosis following resection and clinical and imaging characteristics.

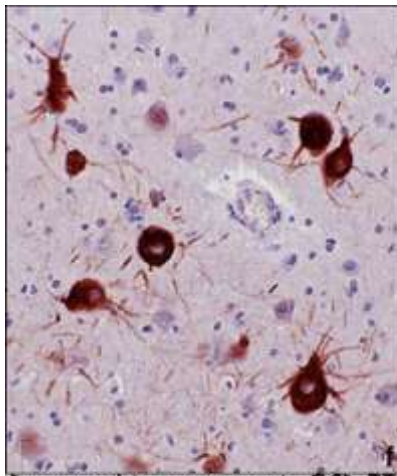
FCD type II neuropathology. The macroscopic appearances from an FCD lesion may show a region of apparent thickening of the grey matter, blurring of the grey-white boundary and the tissue may appear firmer. The overall size of these lesions varies and can be up to several centimetres broad involving both sulci and gyri; occasionally discontinuous regions of dysplasia are noted and in young individuals extensive involvement of the hemisphere may be

Table. ILAE 2011 classification for cortical dysplasia.

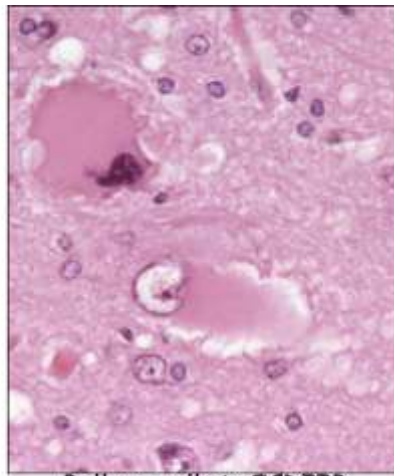
<i>FCD Type I (isolated)</i>	Focal Cortical Dysplasia with abnormal radial cortical lamination (FCD Ia)	Focal Cortical Dysplasia with abnormal tangential cortical lamination (FCD Ib)	Focal Cortical Dysplasia with abnormal radial and tangential cortical lamination (FCD Ic)	
<i>FCD Type II (isolated)</i>	Focal Cortical Dysplasia with dysmorphic neurons (FCD IIa)		Focal Cortical Dysplasia with dysmorphic neurons and balloon cells (FCD IIb)	
<i>FCD Type III (associated with principal lesion)</i>	Focal Cortical Dysplasia in the temporal lobe associated with Hippocampal sclerosis (FCD IIIa)	Focal Cortical Dysplasia adjacent to a glial or glio-neuronal tumor (FCD IIIb)	Focal Cortical Dysplasia adjacent to vascular malformation (FCD IIIc)	Focal Cortical Dysplasia adjacent to any other lesion acquired during early life, e.g., trauma, porencephaly, encephalitis (FCD IIIId)

seen. The region of dysplasia in some cases is not seen on visual inspection. Histological appearances confirm architectural abnormalities of the cortex as common to all types of FCD. An abnormal laminar cortical architecture (dyslamination) is appreciated with indistinct boundaries between cortical layers compared to normal, which is more readily apparent with Nissl or NeuN immunostaining. Cortical layer I may often remain relatively cell-free and defined in the region of dysplasia, but may be broader than normal. The junction between the deep cortical layers and white matter is often ill-defined. A lack of any radial alignment of





Dysmorphic neurones in FCD II
neurofilament positive



Balloon cells in FCDIIB

Profoundly abnormal cortical cell types are present in FCD and define the subtypes. **Dysmorphic neurones** have abnormal size, orientation, dendritic processes and cytoskeletal structure. In Cresyl violet stained sections Nissl appears abnormally clumped and eccentric thickening of nuclear membranes can be seen.

Dysmorphic neurones may be present in any laminar position and are occasionally seen in isolation in otherwise apparently normal cortex adjacent to the main lesion, or in groups trailing into the underlying white matter. In some cases dysmorphic neurones predominate in the pyramidal cell layers (III and V). Abnormal polarity of these neurones ranges from slight rotation to complete inversion in relation to the pial surface⁷⁶. These cells show dysmorphic and extremely tortuous dendrites but with decreased spine density.

Balloon cells have large round soma with a diameter of 20–90 μm or more. The nucleus is eccentric and the cytoplasm pale pink and glassy on H&E (see Figure above). Multinucleate or giant cell forms are frequent. Some balloon cells have vesicular, more centrally-placed nuclei and prominent nucleoli reminiscent of neurones and regarded as indeterminate or transitional cell forms. Following biocytin injection in slice preparations, typical balloon cells lack axons and dendritic spines. They tend to be located in deeper cortical layers, spilling into the white matter but can be present throughout the cortex, particularly layer I. **Giant or hypertrophic pyramidal neurones** retain an overall pyramidal morphology and orientation but are present in any (or throughout all) cortical layers. Biocytin intracellular labelling shows abnormalities of the dendrites of these neurones with thick initial segments, abnormally tortuous but shorter dendrites but with increased branching. The cross sectional area of these neurones (mean 507 μm^2) is significantly larger than normal pyramidal cells⁷⁶. **Immature neurones** are round or oval cells (diameter 10–12 μm) with a thin rim of cytoplasm and rudimentary dendrites. When aggregating in clusters, sometimes mixed with mature neurones in the cortex, they are also referred to as ‘hamartias’⁷⁷.

More recently cytomegalic GABAergic neurones have been identified as a component of FCD⁷⁸. Although these abnormal cell types are instantly recognisable in routine sections, immunohistochemistry allows further characterisation and classification. Neurofilament gene expression has been shown to be augmented in FCD neurones⁷⁹. Striking immunopositivity of dysmorphic and hypertrophic neurones may be observed with neurofilament antibodies in comparison to normal cortex, which highlights their abnormal morphology, alignment and laminar position. In addition, increased density of neurofilament positive processes and threads may be noted in the dysplastic cortex. Over-expression of cytoskeletal proteins may be relevant to the abnormal morphology of dysmorphic neurones as well as their migration, as these proteins are critical to normal axon and dendrite outgrowth and maintenance of the cell

structure. Whether these are primary or secondary effects is not known. In addition, in many abnormal cell types in FCD there is aberrant expression of developmentally-regulated proteins. Strong cytoplasmic positivity of abnormal neurones in FCD is seen with nestin, particularly neurones in deeper layers and heterotopic white matter cells⁸⁰. Balloon cells also show variable expression of nestin, vimentin, GFAP, GFAP delta, doublecortin, neurofilaments, and MAP1B, an immature MAP isoform⁸¹. Membranous expression of stem cell marker CD34 (and CD133 is seen in FCD cases in a proportion of balloon cells located predominantly in the white matter^{82,83}. Co-expression of neuronal and glial markers by abnormal cell types has been shown^{81,84}, confirming aberrant glial-neuronal differentiation and this observation, together with the expression of immature proteins, lend support to a common mal-developmental origin.

Current theories propose FCD represents arrested neuronal migration and differentiation or abnormalities of progenitor cell proliferation and programmed loss of neurones during cortical development. Dysregulation of cell cycle proteins in balloon cells in FCD has been shown⁸⁵, as well as aberrantly expressed apoptotic proteins⁸⁶. For example, it has been suggested that FCD represents remnants of the radial glia network or preserved subplate cells⁸⁷. Quantitative studies in cortical dysplasias reveal an increase in layer I, superficial cortical and white matter neuronal densities in support of a hypothesis of excess neurogenesis and retention of subplate cells late in corticogenesis⁸⁸.

Table. Immunohistochemical studies reported in focal cortical Dysplasia type IIB. IF = intermediate filament.

	Balloon cells	Dysmorphic neurones
Cytoskeletal proteins	GFAP (IF) Vimentin (IF)	NF-H, M (non-phosphorylated) NF-H, L (phosphorylated) MAP2B
Developmentally regulated proteins	Nestin, Vimentin TUJ1 GFAP-delta CD133 CD34 Doublecortin Map1b Pax6	PSA-NCAM Doublecortin Nestin MAP1b Otx1
Aberrant neuronal or glial differentiation	In support of neuronal differentiation: MAP2 Map1b NeuN NF-H	In support of glial differentiation: GFAP expression in some cells
Cell cycle/apoptotic proteins	Bcl-2	
Synaptic proteins		Synaptophysin SV2A (surrounding dysmorphic neurones)

However an overall reduction in cortical neuronal density in FCD IIB cases compared with normal cortex has also been shown, which could indicate a failure of progenitor proliferation but also acquired, ongoing neuronal loss. In any estimates of neuronal density in FCD lesions, the effects of any overall expansion or reduction of volume of the cortical neuropil have to be taken into consideration.

FCD is a sporadic disorder. There are no known family cases of FCD and there is no animal model that precisely resembles the human pathology. One possibility is that FCD may result from a somatic mutation in a precursor or progenitor cell population during development. There has been intense research in order to identify any candidate gene(s) and the resulting affected intracellular pathways, harnessing modern molecular biological techniques on isolated specific abnormal cell types in FCD lesions. Single cell analysis, for example, has shown that the cells within FCD are not clonally derived but probably arise from mixed populations of progenitor cells⁸⁹. Balloon cells have recently been cultured from surgical specimens and display the properties of progenitor cells⁹⁰.

The morphological features of FCD IIB share similarities to those found in the neocortical tubers of the tuberous sclerosis complex (TSC), including dysmorphic, cytomegalic neurones and giant/balloon cells. In fact, in surgical resections, differentiation between these two lesions is often not possible, although it has been quoted that extensive subpial gliosis, dystrophic calcification and dysmyelination is a more common finding in cortical tubers⁹¹. FCD may represent a *forme fruste* or phenotypic variation of TSC and as a clear neuropathological distinction can not yet be reliably made, all patients should be screened for TS if clinically indicated. The pathological similarities between these two lesions has also led to a search for a common genetic link although recent studies argue that TSC and FCD are phenotypically and genetically distinct⁹². TSC is a well defined hereditary tumour syndrome caused by mutations in one of two non-homologous tumour suppressor genes; *TSC1* (9q34) encoding hamartin and *TSC2* (16p13.3) encoding tuberin. Sequence alterations (polymorphisms) in the *TSC1* locus and loss of heterozygosity has been identified in FCD type IIB lesions⁹³ but not in FCD IIA samples⁹⁴. This could implicate a role for TSC genes in balloon-cell containing FCD lesions as distinct from other FCD types. The TSC gene products interact with several intracellular signalling pathways which may be relevant to the pathological features of cortical dysplasia. Likely pathways include activation of Rap1, binding with ezrin (which effects cell adhesion, migration, polarity and cell cycle progression), dysregulation of the insulin signalling mTor/p70SK-S6 pathway (influences cell size and proliferation), and the wnt-1/ β -catenin pathway (implicated in cell survival, cell shape and differentiation, polarity and migration). Molecular studies at present suggest there may be distinct activation of these pathways in FCD IIB and cortical tubers⁹⁵⁻⁹⁸. Furthermore, gene expression studies from giant cells of tubers versus similar balloon cells in FCD show distinctive transcriptional profiles⁹⁶. Histological similarities also exist between FCD and more extensive malformation causing hemimegalencephaly (HME). HME is often sporadic and associated with epilepsy, but occasionally linked with syndromes such as linear sebaceous neavus syndrome, proteous syndrome and rarely TSC. Like FCD, activation of the wnt-1/ β -catenin pathway has been shown in HME⁹⁵. The reelin and cdk5 signalling pathways have also been implicated in the pathogenesis of FCD, as mice deficient in these proteins display marked cortical laminar abnormalities. Finally there is also the alternative but less likely school of thought that FCD is a result of cortical reorganisation and plasticity following a prior cerebral-cortical insult, including early trauma⁹⁹⁻¹⁰¹.

Epilepsy is clearly associated with FCD lesions and how the abnormal cell types might generate seizure activity is under study (for recent review see Wong¹⁰² and Najm et al¹⁰³). Single cell recordings from dysplastic neurones have demonstrated abnormal intrinsic membrane properties and ion channel functions⁷⁶. Using current clamp techniques in *in vitro* slice preparations however, no spontaneous epileptiform depolarisations in cytomegalic neurones were noted, suggesting they are unlikely to operate as ‘pacemaker’ neurones¹⁰⁴. Balloon cells do not display spontaneous synaptic current or action potentials⁷⁶ and lack synaptic contacts¹⁰⁵, suggesting they are relatively inert bystanders. Direct electrocorticographic recordings also support that the centre of the FCD lesion containing balloon cells is less epileptogenic than other regions¹⁰⁶. In these patients the epileptogenic regions mainly reside in the surrounding regions that are dysplastic but relatively devoid of balloon cells. The dysmorphic neurones in FCD show increased expression for glutamate receptor subunits, including NMDA receptors¹⁰⁷. There is evidence for a reduction of number of local inhibitory interneurons and terminals in FCD type II lesions using labelling for GAD^{72,108}, parvalbumin and calbindin^{108,109}. GABA_A receptor subunit mRNAs ($\beta 1$, $\beta 2$, $\alpha 1$, $\alpha 2$) are decreased in abnormal neurones of FCD¹¹⁰, as well as in TSC lesions¹¹¹ and a reduction in GABA transporter 1 (GAT1) has also been shown in FCD¹⁰⁸. Overall these findings would support that a potential lack of inhibitory input may have a role in intrinsic bursting of excitatory neurones. However, other studies propose preserved GABAergic activity in the region of dysplasia¹⁰⁴ and hypertrophic, inhibitory synaptic terminals are often seen to surround dysplastic neurones in ultrastructural studies^{105,108,112} which may represent adaptive changes. Recordings from *in vitro* slice preparations of FCD have also implicated that synchronisation of ictal discharges is in fact initiated by GABAergic mechanisms¹¹³.

Key points regarding balloon cells in FCD:

- Function: electrically inert, absent synaptic input and dendritic spines. Seizure ‘insulating’ maladaptive phenomena?
- Origins: radial glial origin with aberrant differentiation pathways
- Nature: progenitor cell properties
- Proliferative potential: arrest in cell cycle and differentiation/maturation arrest
- Cell size an effect of dysregulate signalling in mTOR and/or PTEN pathway
- Localisation in white matter: associated with hypomyelination.

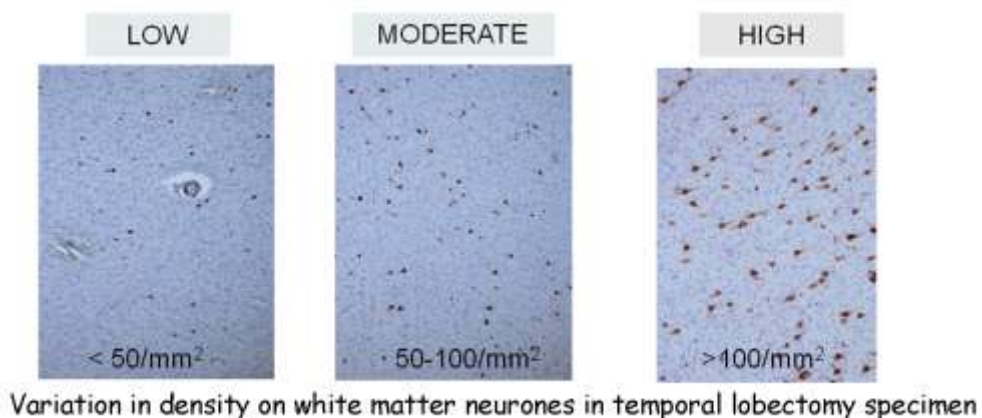
Mild malformations of cortical development

In the Palmini classification scheme⁷², as well as in the new ILAE classification⁷³, mild MCD in epilepsy encompass more subtle cortical abnormalities previously referred to as microdysgenesis or architectural dysplasias. Microdysgenesis has been used over the last two decades, perhaps somewhat vaguely, as a convenient term for a wide range of minor cyto-architectural cortical abnormalities, including indistinct laminar boundaries, cortical neuronal clusters, an excess of layer I and white matter neurones and perivascular oligodendroglia-like cell aggregates among others¹¹⁴⁻¹¹⁶. The diagnosis of microdysgenesis was always clouded with uncertainty regarding the exact criteria and whether all or only some features need be present. In fact different pathological components may have a different aetiology⁹⁴. In the updated Palmini system, mild MCD are divided into two categories: Type I with ectopic neurones placed in or adjacent to layer I; and type II with microscopic neuronal heterotopia outside layer I. More widespread cortical laminar abnormalities are now included under FCD type IA.

Excess single or ‘ectopic’ neurones in layer I in mild MCD type I may have varied origins including residual preplate cells (including reelin-secreting Cajal-Retzius cells¹¹⁷), other early

neurones of the marginal zone, an abnormal persistence of subgranular cell layer neurones, radial migrating GABAergic neurones from the ganglionic eminence, or heterotopic cortical plate neurones. In some cases layer I hypercellularity is readily apparent on qualitative inspection of resected tissues and occasionally forms nodular aggregates. In less obvious cases the use of quantitative analysis to distinguish type I mild MCD from normal cortex remains has been undertaken which may prove of practical value^{88,109,117-119}. As no known immunohistochemical ‘marker’ has been identified that can reliably distinguish ‘ectopic’ from normal layer I neurones, in many cases the diagnosis is difficult.

Similarly with mild MCD type II, which largely refers to ectopic single white matter neurones, there are difficulties in the discrimination from normal interstitial neurones of the white matter, particularly in the temporal lobe where they are more numerous. Possible origin of white matter neurones in mild MCD type II include residual subplate cells or ‘true’ heterotopic cortical plate cells. Whether single neurone white matter heterotopia is pathogenetically related to the more severe MCD of macroscopic nodular heterotopia is unknown. White matter neurones have mixed morphologies and include pyramidal and small inhibitory interneurons and may extend axonal projections to the overlying cortex. Several quantitative studies in patients with temporal lobe epilepsy have been carried out and confirm higher white matter neuronal densities compared to controls (e.g. densities > 8 neurones/2 mm²)¹²⁰ but data vary between studies depending on the types of neurones included and the method of quantitative analysis^{116, 118, 121, 122}. Furthermore, changes to the white matter which influence tissue volume, including atrophy and gliosis, neuronal hypertrophy, and effects of tissue fixation and shrinkage may falsely exaggerate cell counts and need to be considered as potential confounding factors.



FCD type III

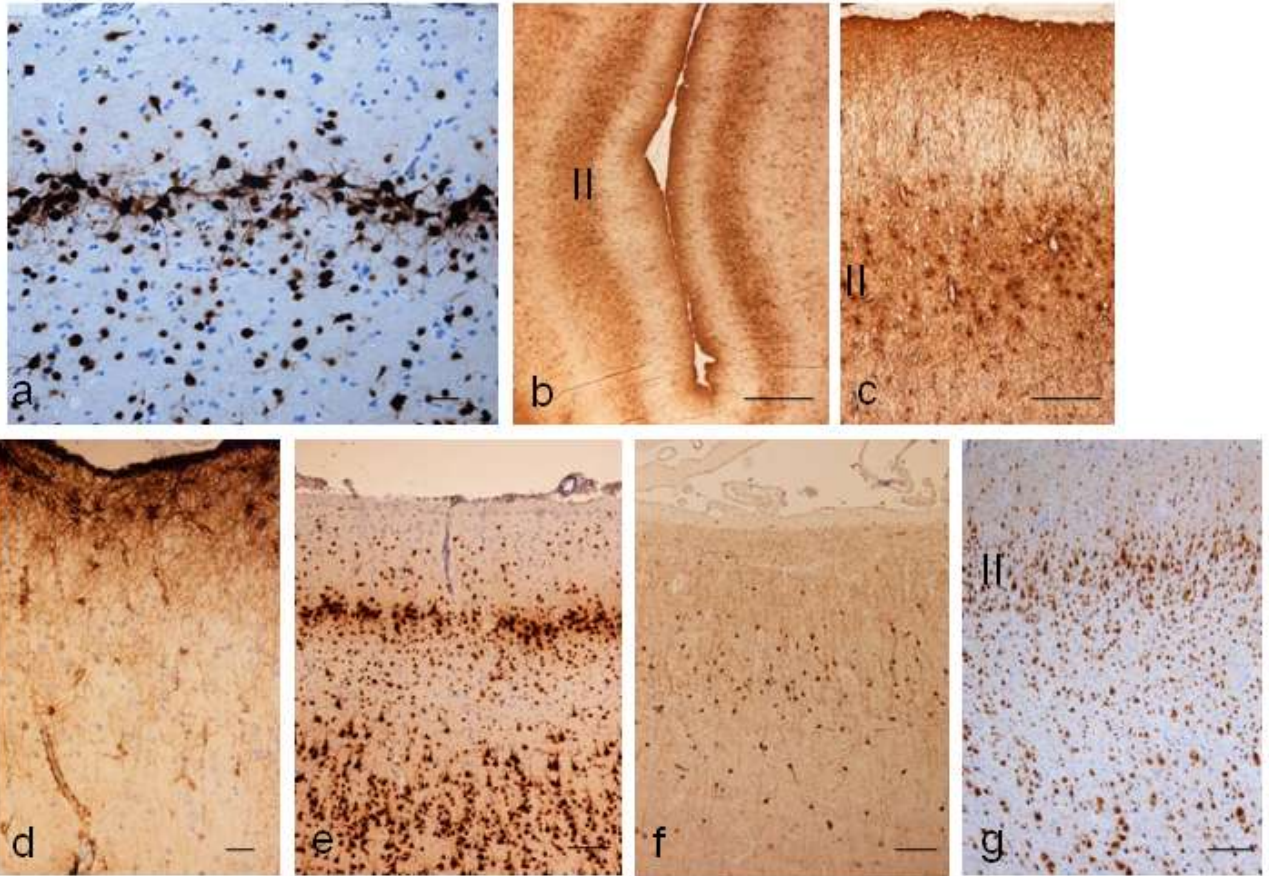
In the revised ILAE classification of cortical dysplasia. FCD type IIIa includes alterations in architectural organisation (cortical dyslamination) and/or cytoarchitectural composition of the temporal lobe (i.e. hypertrophic neurones outside layer V), which occur in patients with mesial temporal sclerosis (MTS, syn. hippocampal sclerosis, Ammon’s horn sclerosis). The aetiology and pathogenesis of FCD type IIIA remains to be determined, but is likely a process associated to MTS. FCD IIIA includes the pathological diagnosis of temporal lobe sclerosis. In MTS patients, an abnormal band of small and clustered ‘granular’ neurones can be observed in approximately 10% of temporal lobe surgical specimens in the outer part of layer II, designated

Table. Studies of quantitative analysis of white matter neuronal number in epilepsy (adapted from Blumcke et al⁴²).

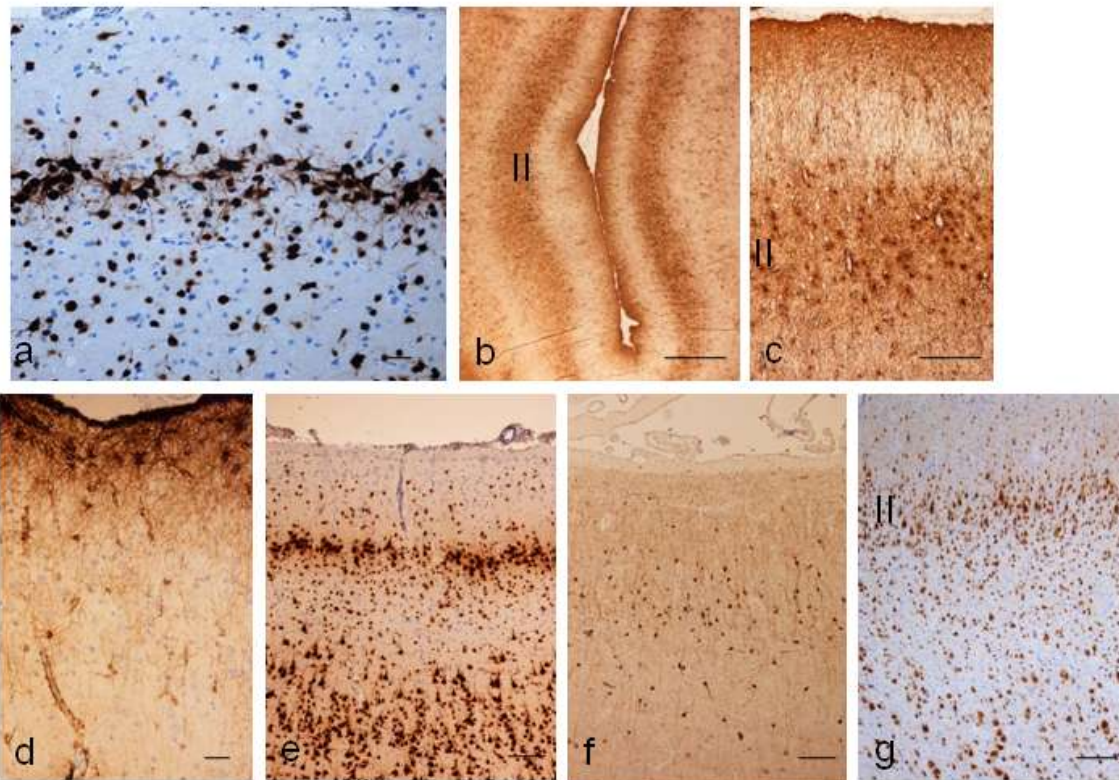
Study Reference	Pathology (n = number of cases)	Preparation	Method	Result in epilepsy (control values if tested)
Nissl stain, profile cell counting (2D) in TLE				
¹²⁰	Temporal lobe epilepsy (n = 49)	Nissl	Profile counting (2D) 'deep' white matter	4/mm ² (No controls >4/mm ²)*
¹²¹	Temporal lobe epilepsy (n = 22)	Nissl	Profile counting (2D) All TL white matter	4.11/mm ² * (2.35/mm ²)
Nissl stain stereology (3D) in TLE				
¹¹⁸	ATL adjacent to HS (n = 31)	Nissl (cresyl violet)	Stereology (3D) All white matter	1160/mm ³
¹²²	ATL adjacent to HS (50%) (Brodmann area 36) (n = 10)	Nissl	Stereology (3D) All white matter	1010/mm ³ *
Immunostained sections cell profile counts (2D)				
¹²³	FCD I and II (no difference between type I and II) (n = 25)	MAP2 on 4 micron thick sections	Cell profile (2D) 1 mm ³ of deep white matter	21.4±6.8/mm ² (10.5±2.9/mm ²)* Controls
¹²⁴	ATL adjacent to HS (n = 10)	NeuN on 7 micron thick sections	Cell profile (2D) ROI in deep white matter	23.4/mm ² (No control group)

as temporal lobe sclerosis (TLS)⁵⁷. TLS is likely to represent severe neuronal cell loss in layer II and III with associated laminar gliosis (GFAP-positive astroglia; see Figure over). This may have been acquired early in life following an initial precipitating injury (IPI) as a febrile seizure (for review see ref Thom et al⁵⁷). Horizontal bundles of myelinated axons can be observed to a variable degree in all cases using HE-LFB stainings. There is regional variability affecting the temporal lobe in 40% of MTS patients, whereas extensive involvement throughout the temporal lobe occurs in 20%. There is no correlation between this FCD variant and MRI findings in these patients.

FCD type IIIb includes dysplasias adjacent to glioneuronal tumours (see section below).



Patterns of neuronal loss and gliosis in temporal lobe sclerosis adjacent to Hippocampal sclerosis in a patient with MTLE. a = reorganisation and clustering of residual neurones in layer II, b,c= superficial gliosis in layer II, d= comparison control gliosis, e=clustering of pyramidal neurones in layer II and f=same case with normal distribution of inhibitory neurones in superficial cortex. g=FCDIIB with similar cell packing in layer II



Patterns of neuronal loss and gliosis in temporal lobe sclerosis adjacent to Hippocampal sclerosis in a patient with MTLE. a = reorganisation and clustering of residual neurones in layer II, b,c= superficial gliosis in layer II, d= comparison control gliosis, e=clustering of pyramidal neurones in layer II and f=same case with normal distribution of inhibitory neurones in superficial cortex. g=FCBIIB with similar cell packing in layer II

Tumours and epilepsy

A wide variety of tumour types, particularly where there is cortical extension, can manifest clinically with focal seizures. Within this group, patients with longer histories of early onset, focal or partial epilepsy, neuroimaging and pathological studies more often identify cortically-based, slow-growing tumours. In many of these cases the main aim of surgical treatment is seizure control rather than to halt any tumour progression and low grade glial or mixed glioneuronal tumours are common diagnoses in large surgical series. In the section below we focus on the possible mechanisms of their intrinsic epileptogenicity in relation to their distinctive cell composition, as well as their developmental origins and links with cortical dysplasias.

Glioneuronal tumours

There are two main tumour types in this category, dysembryoplastic neuroepithelial tumours (DNT) and gangliogliomas. The relative frequency of these lesions varies between epilepsy centres; in some centres DNT are more commonly diagnosed and in others gangliogliomas^{125,126}. Both tumour types have a predilection for the temporal lobe. Other glial tumours associated with epilepsy may also have a minor neuronal component, including pleomorphic xanthoastrocytoma, and pilocytic astrocytoma. In addition, newer forms of glial tumours, such as the isomorphic astrocytoma¹²⁷, the angiocentric neuroepithelial tumour or glioma¹²⁸ and new glioneuronal tumours such as the papillary glioneuronal tumour¹²⁹ continue to be recognised, some strongly associated with long standing epilepsy¹³⁰.

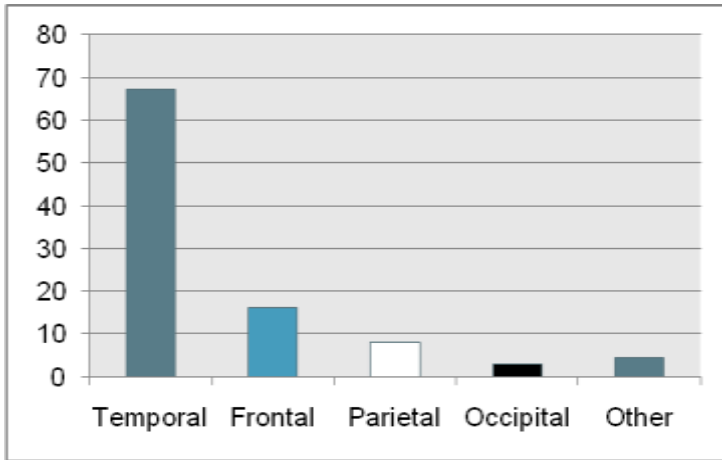


Figure. Bar chart of 624 DNT reported in literature showing localisation (%).

DNT are clinically associated with various types of partial seizures. The pathological features of the classical DNT are characterised by a multinodular, intracortical architecture composed of cells with mixed cytological features^{131,132}. The predominant cell type is the oligodendrocyte-like cell (OLC) but regions with an astrocytic-piloid growth pattern, including eosinophilic granular bodies and Rosenthal fibres, are commonly seen. Among the glial cells is a mature neuronal component and in some cases single neurones suspended within a myxoid matrix between surrounding OLC (the ‘glioneuronal element’ – see Figure below).

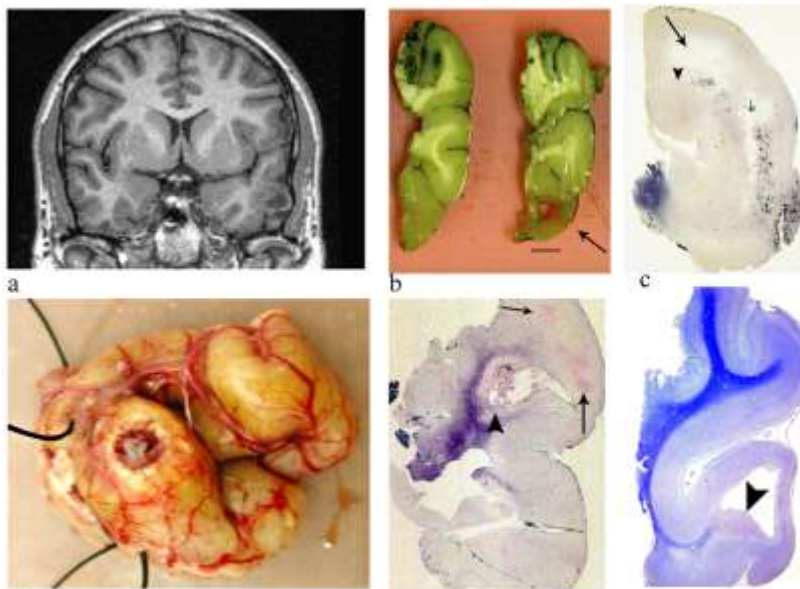


Figure. Pathology features of DNT a= MRI features of temporal lobe tumour b= cystic tumour in inferior gyrus (arrow), c= tumour diffusely infiltrating cortex d= calcified DNT abutting cortical surface, e= multinodular DNT, f= section through a cystic DNT.

These neurones display minimal cytological atypia and immunophenotypically resemble neurones of adjacent cortex, including expression of mature neuronal markers as MAP2 and NeuN and lack of expression of immature markers as nestin and CD34. This classical form of DNT is readily recognised, particularly in large lobectomy specimens in contrast to small biopsies where a precise pathological diagnosis is less feasible due to the marked heterogeneity of these lesions.

Other common histological forms of DNT encountered in large epilepsy series are the single nodular and diffuse forms^{132,133}. The latter have a similar cytological composition but show more diffuse infiltration of the cortex and white matter. All DNT may show cystic degeneration, calcification, pigmentation, leptomeningeal extension and nuclear pleomorphism of OLC, but mitotic figures and necrosis are rare. Resection typically results in greatly improved seizure control and these lesions tend not to recur^{132,134}, although there are some recent reports documenting recurrent and rare malignant transformation¹³⁵⁻¹³⁷.

Gangliogliomas are tumours with a biphasic cytopathology composed of dysmorphic neurones (abnormally clustered, localised and cytomegalic neurones) and a glial component which may be astrocytic or oligodendroglial in morphology¹²⁶. A major diagnostic difficulty is the distinction of diffusely infiltrating low-grade cortical glial tumour from a ganglioglioma. Atypical neurones may also be a rare observation in otherwise typical DNT¹³² and there are reports of glioneuronal tumours showing hybrid features between DNT and ganglioglioma^{138,139} suggesting common biological links between these entities. Sequence alterations in TSC2 gene and involvement of the reelin signalling pathway may be important in the aetiology of gangliogliomas as in FCD^{140,141}. Expression of stem cell epitope, CD34, has also been noted in up to 80% of gangliogliomas in the small cell component¹²⁶, which may be of diagnostic value. Predominantly gangliogliomas are grade I neoplasms associated with a good outcome. Over 80% of patients become seizure free following surgery, and only a small percentage of cases show tumour recurrence (1–3%) or represent WHO grade II or III lesions (2–6%) which warrant closer follow up^{126,134,142}.

The exact mechanisms resulting in hyperexcitability by tumours remain to be delineated in these lesions¹²⁶. The prominent neuronal component of these tumours is one obvious explanation for their potent epileptogenicity. Studies suggest expression of several glutamate receptor subtypes on both the neuronal and glial component¹⁴³. It is also considered that the OLC of DNT represent immature neuronal cell populations¹⁴⁴; immunophenotypically these cells are typically GFAP negative, do not express mature neuronal markers MAP2 and NeuN^{145,146} but are nestin positive. In addition, laser microdissection of the CD34-expressing small cell component of gangliogliomas confirms neuronal rather than glial differentiation in these cells¹⁴⁷. It is therefore possible that the immature/small cell component of glio-neuronal tumours is contributing to pro-epileptogenic circuits.

DNT, as well as gangliogliomas, have often been reported in association with adjacent cortical dysplasia. The descriptions of the dysplasia from reported studies mainly correlates with FCD type IA (disturbed lamination/dyslaminar) and MCD type I (layer I hypercellularity or ‘subpial cellular band’)^{131,132,148} using the current Palmini system⁷². Severe types of cortical dysplasia, such as FCD II, are rarely reported with DNT¹³². The diagnosis of additional cortical dysplasia should naturally be distinguished from the disturbance of the cortical architecture due to diffuse tumour infiltration by OLC. Furthermore ‘hamartia’ like clusters of OLC, mixed with neurones, forming aggregates around 0.2–1.0 mm (also called ‘microdysgenetic nodules’) are also common findings adjacent to both DNT and gangliogliomas⁷⁷. They may represent

precursor lesions of these tumours and may escape detection on routine H&E but are readily observed on immunolabelling for stem cell marker CD34, particularly when in the temporal lobe^{126,149}. It has also been speculated that DNT arise from the persistence of immature cells of the subpial granular cell layer with capacity for divergent differentiation¹³¹. The identification of cortical dysplasia in the vicinity of glioneuronal tumours raises the question not only of common biological origins but, importantly, where the intrinsic epileptogenicity arises – lesion or perilesional cortex. Intracerebral recordings from electrodes suggest that ictogenesis resides in the ganglioglioma itself¹²⁶. However, high expression of connexin 43 and 32 gap-junction proteins has been shown in the perilesional astrocytes in the adjacent cortex of epilepsy-associated glio-neuronal tumours which may contribute to seizures¹⁵⁰. Establishing more precisely where the seizures arise will have important relevance to the surgical management of these lesions.

Vascular malformations in epilepsy surgery

Vascular malformations form up to 10% of lesions encountered in epilepsy surgical series and the main types are arteriovenous malformations (AVM) and cavernomas, with telangiectatic or angiodysgenetic lesions more rarely encountered. Although regarded as congenital, these lesions are dynamic and may even rarely arise de novo. Epilepsy is a common presenting clinical feature in 17% of AVM compared to spontaneous cerebral haemorrhage (in 43%) and headache (25%)¹⁵¹ and is the most common presenting symptom in cavernomas (79%). Seizures may be generalised or partial¹⁵². Vascular malformations may occur at any site in the CNS and vary greatly in size and extent and cavernomas may be multiple, particularly in the rarer hereditary forms. Common features to both AVM and cavernomas include extensive perilesional gliosis and tissue microhaemorrhages indicative of sub-clinical bleeds. Early surgery is currently regarded as the optimal treatment both for the epilepsy and to reduce the risk of further haemorrhage with 84% of patients with cavernomas becoming seizure free¹⁵². The possible mechanisms inducing epilepsy include local ischaemia as a result of arteriovenous shunting, the marked associated peripheral gliosis, haemosiderin deposition or secondary epileptogenesis occurring in the temporal lobe. Extended lesionectomy, to include the gliotic margin, may be carried out particularly where pre-operative investigations including electrocorticography (EcoG) and magnetoencephalogram (MEG) support that the epileptic activity resides in the adjacent tissue¹⁵³, although this has to be balanced with the increased risk of surgical complications¹⁵¹.

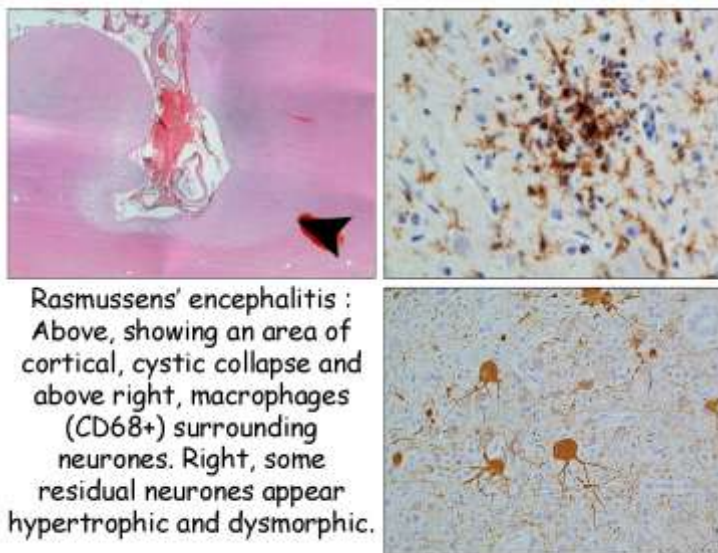
Hamartomas in epilepsy surgery

Hamartomas in epilepsy are a poorly defined pathological group forming a small number of cases in different epilepsy surgical series. **Glio-neuronal hamartomas** have been described in various cortical locations, particularly temporal and frontal lobes, composed of circumscribed masses of mature but haphazardly arranged cell types, sometimes reported in association with adjacent cortical dysplasia¹⁵⁴. The imaging characteristics of glio-neuronal hamartomas are variable^{155,156}; their lack of growth and mitotic activity help to distinguish these lesions from low grade tumours and they tend not to recur¹⁵⁴. The **hypothalamic hamartoma** has a strong association with intrinsic subcortical epileptogenesis and gelastic seizures and may be associated with the development of secondary cortical epileptogenesis¹⁵⁷. Unlike the tuberous sclerosis complex, hamartomas or malformative cortical lesions are relatively rarely reported in neurofibromatosis type 1 (NF1)¹⁵⁸, a syndrome in which epilepsy occurs in up to 6% of patients¹⁵⁹. NF2 can be associated with multiple cortical glial-microhamartomas that are often incidental findings at post mortem. Interestingly, the presumed hamartomatous cell

proliferation of **meningioma** (MA), when associated with the NF2 complex, does not clinically manifest with seizures whereas in sporadic MA over 80% of patients present with epilepsy¹⁶⁰. The more common sporadic form of MA is typically solitary and EEG suggests the epileptogenicity is confined to the adjacent cortex; seizures may persist in over half of patients following surgical treatment¹⁶¹.

Rasmussen's encephalitis

Rasmussen's encephalitis (RE) is a rare sporadic syndrome of unknown aetiology typically presenting in childhood with intractable seizures and associated with progressive unilateral hemispheric atrophy and neurological deficit. The severity of the inflammatory process and the extent of the cortical scarring vary with the duration of the disease process and traditionally has been divided into four stages. The early stages (1 and 2) are characterised by more active chronic inflammation and the later stages (3 and 4) with less active inflammation and more extensive scarring¹⁶². Inflammatory infiltrates in the cortex consist mainly of T lymphocytes (CD8>CD4+) with perivascular and perineuronal clusters¹⁶³. B lymphocytes are less frequently present in the perivascular cuffs and plasma cells are rare. Widespread activation of microglia may be seen, as well as microglial clusters and nodules (see Figure below), but macrophage infiltrates are less common. Patchy neuronal degeneration, neuronophagia and neuronal dropout are present in the early stages.



With progressive damage, neuronal ballooning with distortion of cell shape, neurofilament accumulation, and laminar disorganisation may also be noted, reminiscent of cortical dysplasia¹⁶² and apoptotic neurones have been identified¹⁶³. In the later stages large areas of pan-laminar or patchy cortical necrosis are characteristic with extensive neuronal loss, astrocytic gliosis and cortical spongiosis and the inflammatory process is less prominent. Cortical scars may be extensive, involving a whole gyrus or more 'punched out' wedge-like areas of destruction may be observed. The topography of the inflammatory process varies within specimens with regions of either atrophy, active inflammation alternating with stretches of uninvolved cortex. The multifocal nature of the disease process highlights why cortical biopsies may give a false negative result. Patchy inflammation and myelin loss in the

underlying white matter and involvement of the deep grey nuclei may also be present in RE and inflammation may extend to the hippocampus and additional hippocampal sclerosis may be present. In cases where post mortem tissue is available, true bilateral disease with associated inflammatory change is probably very rare^{164, 165}.

References

1. HAUT SR, VELISKOVA J, MOSHE SL. Susceptibility of immature and adult brains to seizure effects. *Lancet Neurol* 2004;3:608-617.
2. BLUMCKE I. Neuropathology of focal epilepsies: a critical review. *Epilepsy Behav* 2009;15:34-39.
3. LIOU AK, CLARK RS, HENSHALL DC, YIN XM, CHEN J. To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-activated signaling pathways and apoptotic pathways. *Prog Neurobiol* 2003;69:103-142.
4. EKDAHL CT, ZHU C, BONDE S, BAHR BA, BLOMGREN K, LINDVALL O. Death mechanisms in status epilepticus-generated neurons and effects of additional seizures on their survival. *Neurobiol Dis* 2003;14:513-523.
5. SUTULA TP, PITKANEN A. More evidence for seizure-induced neuron loss: is hippocampal sclerosis both cause and effect of epilepsy? *Neurology* 2001;57:169-170.
6. MEN S, LEE DH, BARRON JR, MUNOZ DG. Selective neuronal necrosis associated with status epilepticus: MR findings. *AJNR Am J Neuroradiol* 2000;21:1837-1840.
7. POHLMANN-EDEN B, GASS A, PETERS CN, WENNBERG R, BLUMCKE I. Evolution of MRI changes and development of bilateral hippocampal sclerosis during long lasting generalised status epilepticus. *J Neurol Neurosurg Psychiatry* 2004;75:898-900.
8. CORSELLIS JA, BRUTON CJ. Neuropathology of status epilepticus in humans. *Adv Neurol* 1983;34:129-139.
9. TSUCHIDA TN, BARKOVICH AJ, BOLLEN AW, HART AP, FERRIERO DM. Childhood status epilepticus and excitotoxic neuronal injury. *Pediatr Neurol* 2007;36:253-257.
10. FUJIKAWA DG, ITABASHI HH, WU A, SHINMEI SS. Status epilepticus-induced neuronal loss in humans without systemic complications or epilepsy. *Epilepsia* 2000;41:981-991.
11. TAN N, URICH H. Postictal cerebral hemiatrophy: with a contribution to the problem of crossed cerebellar atrophy. *Acta Neuropathol (Berl)* 1984;62:332-339.
12. THOM M, ZHOU J, MARTINIAN L, SISODIYA S. Quantitative post-mortem study of the hippocampus in chronic epilepsy: seizures do not inevitably cause neuronal loss. *Brain* 2005;128:1344-1357.
13. LAWN ND, BAMLET WR, RADHAKRISHNAN K, O'BRIEN PC, SO EL. Injuries due to seizures in persons with epilepsy: a population-based study. *Neurology* 2004;63:1565-1570.
14. KLOSTER R, ENGELSKJON T. Sudden unexpected death in epilepsy (SUDEP): a clinical perspective and a search for risk factors. *J Neurol Neurosurg Psychiatry* 1999;67:439-444.
15. SHIELDS LB, HUNSAKER DM, HUNSAKER JC 3rd, PARKER JC Jr. Sudden unexpected death in epilepsy: neuropathologic findings. *Am J Forensic Med Pathol* 2002;23:307-314.
16. BLACK M, GRAHAM DI. Sudden unexplained death in adults caused by intracranial pathology. *J Clin Pathol* 2002;55:44-50.
17. OPEKIN K, THOMAS A, BERKOVIC SF. Does cardiac conduction pathology contribute to sudden unexpected death in epilepsy? *Epilepsy Res* 2000;40:17-24.
18. DAVIS GG, MCGWIN G Jr. Comparison of heart mass in seizure patients dying of sudden unexplained death in epilepsy to sudden death due to some other cause. *Am J Forensic Med Pathol* 2004;25:23-28.
19. NATELSON BH, SUAREZ RV, TERRENCE CF, TURIZO R. Patients with epilepsy who die suddenly have cardiac disease. *Arch Neurol* 1998;55:857-860.
20. P-CODREA TIGARAN S, DALAGER-PEDERSEN S, BAANDRUP U, DAM M, VESTERBY-CHARLES A. Sudden unexpected death in epilepsy: is death by seizures a cardiac disease? *Am J Forensic Med Pathol* 2005;26:99-105.
21. DE LA GRANDMAISON GL. Is there progress in the autopsy diagnosis of sudden unexpected death in adults? *Forensic Sci Int* 2006;156:138-144.
22. WILLIAMS J, LAWTHOM C, DUNSTAN FD et al. Variability of antiepileptic medication taking behaviour in sudden unexplained death in epilepsy: hair analysis at autopsy. *J Neurol Neurosurg Psychiatry* 2006;77:481-484.
23. TU E, BAGNALL RD, DUFLOU J, SEMSARIAN C. Post-mortem review and genetic analysis of sudden unexpected death in epilepsy (SUDEP) cases. *Brain Pathol* 2011;21:201-208.
24. TOMSON T, NASHEF L, RYVLIN P. Sudden unexpected death in epilepsy: current knowledge and future directions. *Lancet Neurol* 2008;7:1021-1031.
25. LATHERS CM. Epilepsy and sudden death: personal reflections and call for global action. *Epilepsy Behav* 2009;15:269-277.
26. ENGEL J Jr, WIEBE S, FRENCH J et al. Practice parameter: temporal lobe and localized neocortical resections for epilepsy: report of the Quality Standards Subcommittee of the American Academy of Neurology, in association with the American Epilepsy Society and the American Association of Neurological Surgeons. *Neurology* 2003;60:538-547.
27. LHATOO SD, SOLOMON JK, MCEVOY AW, KITCHEN ND, SHORVON SD, SANDER JW. A prospective study of the requirement for and the provision of epilepsy surgery in the United Kingdom. *Epilepsia* 2003;44:673-676.

28. BLUMCKE I, BECK H, LIE AA, WIESTLER OD. Molecular neuropathology of human mesial temporal lobe epilepsy. *Epilepsy Res* 1999;36:205-223.
29. BLUMCKE I. Neuropathology of focal epilepsies: A critical review. *Epilepsy Behav* 2009;15:476-480.
30. DE LANEROLLE NC, LEE TS. New facets of the neuropathology and molecular profile of human temporal lobe epilepsy. *Epilepsy Behav* 2005;7:190-203.
31. THOM M. Hippocampal sclerosis: progress since Sommer. *Brain Pathol* 2009;19:565-572.
32. DAVIES KG, HERMANN BP, DOHAN FC Jr, FOLEY KT, BUSH AJ, WYLER AR. Relationship of hippocampal sclerosis to duration and age of onset of epilepsy, and childhood febrile seizures in temporal lobectomy patients. *Epilepsy Res* 1996;24:119-126.
33. BLUMCKE I, PAULI E, CLUSMANN H et al. A new clinico-pathological classification system for mesial temporal sclerosis. *Acta Neuropathol (Berl)* 2007;113:235-244.
34. THOM M, LIAGKOURAS I, ELLIOT KJ et al. Reliability of patterns of hippocampal sclerosis as predictors of postsurgical outcome. *Epilepsia* 2010;51:1801-1808.
35. HOUSER CR. Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res* 1990;535:195-204.
36. LURTON D, EL BAHH B, SUNDSTROM L, ROUGIER A. Granule cell dispersion is correlated with early epileptic events in human temporal lobe epilepsy. *J Neurol Sci* 1998;154:133-136.
37. LURTON D, SUNDSTROM L, BRANA C, BLOCH B, ROUGIER A. Possible mechanisms inducing granule cell dispersion in humans with temporal lobe epilepsy. *Epilepsy Res* 1997;26:351-361.
38. WIESER HG. ILAE Commission Report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia* 2004;45:695-714.
39. THOM M, SISODIYA SM, BECKETT A et al. Cytoarchitectural abnormalities in hippocampal sclerosis. *J Neuropathol Exp Neurol* 2002;61:510-519.
40. SHAPIRO LA, RIBAK CE. Integration of newly born dentate granule cells into adult brains: hypotheses based on normal and epileptic rodents. *Brain Res Brain Res Rev* 2005;48:43-56.
41. EL BAHH B, LESPINET V, LURTON D, COUSSEMACEQ M, LE GAL LA SALLE G, ROUGIER A. Correlations between granule cell dispersion, mossy fiber sprouting, and hippocampal cell loss in temporal lobe epilepsy. *Epilepsia* 1999;40:1393-1401.
42. BLUMCKE I, KISTNER I, CLUSMANN H et al. Towards a clinico-pathological classification of granule cell dispersion in human mesial temporal lobe epilepsies. *Acta Neuropathol* 2009;117:535-544.
43. SUZUKI F, HEINRICH C, BOEHRER A et al. Glutamate receptor antagonists and benzodiazepine inhibit the progression of granule cell dispersion in a mouse model of mesial temporal lobe epilepsy. *Epilepsia* 2005;46:193-202.
44. GEBHARDT C, DEL TURCO D, DRAKEW A et al. Abnormal positioning of granule cells alters afferent fiber distribution in the mouse fascia dentata: morphologic evidence from reeler, apolipoprotein E receptor 2-, and very low density lipoprotein receptor knockout mice. *J Comp Neurol* 2002;445:278-292.
45. FROTSCHER M, HAAS CA, FORSTER E. Reelin controls granule cell migration in the dentate gyrus by acting on the radial glial scaffold. *Cereb Cortex* 2003;13:634-640.
46. HAAS CA, FROTSCHER M. Reelin deficiency causes granule cell dispersion in epilepsy. *Exp Brain Res* 2010;200:141-149.
47. CAVAZOS JE, ZHANG P, QAZI R, SUTULA TP. Ultrastructural features of sprouted mossy fiber synapses in kindled and kainic acid-treated rats. *J Comp Neurol* 2003;458:272-292.
48. THOM M, MARTINIAN L, CATARINO C et al. Bilateral reorganization of the dentate gyrus in hippocampal sclerosis: a postmortem study. *Neurology* 2009;73:1033-1040.
49. SUTULA T, CASCINO G, CAVAZOS J, PARADA I, RAMIREZ L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol* 1989;26:321-330.
50. YILMAZER-HANKE DM, WOLF HK, SCHRAMM J, ELGER CE, WIESTLER OD, BLUMCKE I. Subregional pathology of the amygdala complex and entorhinal region in surgical specimens from patients with pharmacoresistant temporal lobe epilepsy. *J Neuropathol Exp Neurol* 2000;59:907-920.
51. NIITTYKOSKI M, NISSINEN J, PENTTONEN M, PITKANEN A. Electrophysiologic changes in the lateral and basal amygdaloid nuclei in temporal lobe epilepsy: an in vitro study in epileptic rats. *Neuroscience* 2004;124:269-281.
52. BERNASCONI N, ANDERMANN F, ARNOLD DL, BERNASCONI A. Entorhinal cortex MRI assessment in temporal, extratemporal, and idiopathic generalized epilepsy. *Epilepsia* 2003;44:1070-1074.
53. DE GUZMAN P, D'ANTUONO M, AVOLI M. Initiation of electrographic seizures by neuronal networks in entorhinal and perirhinal cortices in vitro. *Neuroscience* 2004;123:875-886.
54. DU F, WHETSELL WO Jr, ABOU-KHALIL B, BLUMENKOPF B, LOTHMAN EW, SCHWARCZ R. Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. *Epilepsy Res* 1993;16:223-233.
55. DU F, EID T, LOTHMAN EW, KOHLER C, SCHWARCZ R. Preferential neuronal loss in layer III of the medial entorhinal cortex in rat models of temporal lobe epilepsy. *J Neurosci* 1995;15:6301-6313.
56. DAWODU S, THOM M. Quantitative neuropathology of the entorhinal cortex region in patients with hippocampal sclerosis and temporal lobe epilepsy. *Epilepsia* 2005;46:23-30.
57. THOM M, ERIKSSON S, MARTINIAN L et al. Temporal lobe sclerosis associated with hippocampal sclerosis in temporal lobe epilepsy: neuropathological features. *J Neuropathol Exp Neurol* 2009;68:928-938.

58. BLANC F, MARTINIAN L, LIAGKOURAS I, CATARINO C, SISODIYA SM, THOM M. Investigation of widespread neocortical pathology associated with hippocampal sclerosis in epilepsy: a postmortem study. *Epilepsia* 2011;52:10-21.
59. DEASY NP, JAROSZ JM, ELWES RC, POLKEY CE, COX TC. Thalamic changes with mesial temporal sclerosis: MRI. *Neuroradiology* 2000;42:346-351.
60. MARGERISON JH, CORSELLIS JA. Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain* 1966;89:499-530.
61. BERTRAM EH, ZHANG D, WILLIAMSON JM. Multiple roles of midline dorsal thalamic nuclei in induction and spread of limbic seizures. *Epilepsia* 2008;49:256-268.
62. HAGEMANN G, LEMIEUX L, FREE SL et al. Cerebellar volumes in newly diagnosed and chronic epilepsy. *J Neurol* 2002;249:1651-1658.
63. HERMANN BP, BAYLESS K, HANSEN R, PARRISH J, SEIDENBERG M. Cerebellar atrophy in temporal lobe epilepsy. *Epilepsy Behav* 2005;7:279-287.
64. CROOKS R, MITCHELL T, THOM M. Patterns of cerebellar atrophy in patients with chronic epilepsy: a quantitative neuropathological study. *Epilepsy Res* 2000;41:63-73.
65. TEIXEIRA RA, LI LM, SANTOS SL, ZANARDI VA, GUERREIRO CA, CENDES F. Crossed cerebellar atrophy in patients with precocious destructive brain insults. *Arch Neurol* 2002;59:843-847.
66. OHMORI H, OGURA H, YASUDA M et al. Developmental neurotoxicity of phenytoin on granule cells and Purkinje cells in mouse cerebellum. *J Neurochem* 1999;72:1497-1506.
67. TAUER U, KNOTH R, VOLK B. Phenytoin alters Purkinje cell axon morphology and targeting in vitro. *Acta Neuropathol (Berl)* 1998;95:583-591.
68. HERMANN B, SEIDENBERG M, SEARS L et al. Cerebellar atrophy in temporal lobe epilepsy affects procedural memory. *Neurology* 2004;63:2129-2131.
69. SANDOK EK, O'BRIEN TJ, JACK CR, SO EL. Significance of cerebellar atrophy in intractable temporal lobe epilepsy: a quantitative MRI study. *Epilepsia* 2000;41:1315-1320.
70. SPECHT U, MAY T, SCHULZ R et al. Cerebellar atrophy and prognosis after temporal lobe resection. *J Neurol Neurosurg Psychiatry* 1997;62:501-506.
71. PITKANEN A, LUKASIUK K. Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy Behav* 2009;14 Suppl 1:16-25.
72. PALMINI A, NAJM I, AVANZINI G et al. Terminology and classification of the cortical dysplasias. *Neurology* 2004;62:S2-8.
73. BLUMCKE I, THOM M, ARONICA E et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia* 2011;52:158-174.
74. FAUSER S, SCHULZE-BONHAGE A, HONEGGER J et al. Focal cortical dysplasias: surgical outcome in 67 patients in relation to histological subtypes and dual pathology. *Brain* 2004;127:2406-2418.
75. HILDEBRANDT M, PIEPER T, WINKLER P, KOLODZIEJCZYK D, HOLTHAUSEN H, BLUMCKE I. Neuropathological spectrum of cortical dysplasia in children with severe focal epilepsies. *Acta Neuropathol (Berl)* 2005;110:1-11.
76. CEPEDA C, HURST RS, FLORES-HERNANDEZ J et al. Morphological and electrophysiological characterization of abnormal cell types in pediatric cortical dysplasia. *J Neurosci Res* 2003;72:472-486.
77. BLUMCKE I, LOBACH M, WOLF HK, WIESTLER OD. Evidence for developmental precursor lesions in epilepsy-associated glioneuronal tumors. *Microsc Res Tech* 1999;46:53-58.
78. ANDRE VM, WU N, YAMAZAKI I et al. Cytomegalic interneurons: a new abnormal cell type in severe pediatric cortical dysplasia. *J Neuropathol Exp Neurol* 2007;66:491-504.
79. TAYLOR JP, SATER R, FRENCH J, BALTUCH G, CRINO PB. Transcription of intermediate filament genes is enhanced in focal cortical dysplasia. *Acta Neuropathol (Berl)* 2001;102:141-148.
80. CRINO PB, TROJANOWSKI JQ, EBERWINE J. Internexin, MAP1B, and nestin in cortical dysplasia as markers of developmental maturity. *Acta Neuropathol (Berl)* 1997;93:619-627.
81. ENGLUND C, FOLKERTH RD, BORN D, LACY JM, HEVNER RF. Aberrant neuronal-gial differentiation in Taylor-type focal cortical dysplasia (type IIA/B). *Acta Neuropathol (Berl)* 2005;109:519-533.
82. FAUSER S, BECKER A, SCHULZE-BONHAGE A et al. CD34-immunoreactive balloon cells in cortical malformations. *Acta Neuropathol (Berl)* 2004;108:272-278.
83. YING Z, GONZALEZ-MARTINEZ J, TILELLI C, BINGAMAN W, NAJM I. Expression of Neural Stem Cell Surface Marker CD133 in Balloon Cells of Human Focal Cortical Dysplasia. *Epilepsia* 2005;46:1716-1723.
84. TASSI L, PASQUIER B, MINOTTI L et al. Cortical dysplasia: electroclinical, imaging, and neuropathologic study of 13 patients. *Epilepsia* 2001;42:1112-1123.
85. THOM M, MARTINIAN L, SEN A et al. An investigation of the expression of G1-phase cell cycle proteins in focal cortical dysplasia type IIB. *J Neuropathol Exp Neurol* 2007;66:1045-1055.
86. CHAMBERLAIN WA, PRAYSON RA. Focal cortical dysplasia type II (malformations of cortical development) aberrantly expresses apoptotic proteins. *Appl Immunohistochem Mol Morphol* 2008;16:471-476.
87. CEPEDA C, ANDRE VM, LEVINE MS et al. Epileptogenesis in pediatric cortical dysplasia: the dysmature cerebral developmental hypothesis. *Epilepsy Behav* 2006;9:219-235.

88. ANDRES M, ANDRE VM, NGUYEN S et al. Human cortical dysplasia and epilepsy: an ontogenetic hypothesis based on volumetric MRI and NeuN neuronal density and size measurements. *Cereb Cortex* 2005;15:194-210.
89. HUA Y, CRINO PB. Single cell lineage analysis in human focal cortical dysplasia. *Cereb Cortex* 2003;13:693-699.
90. YASIN SA, LATAK K, BECHERINI F et al. Balloon cells in human cortical dysplasia and tuberous sclerosis: isolation of a pathological progenitor-like cell. *Acta Neuropathol* 2010;120:85-96.
91. MACKAY MT, BECKER LE, CHUANG SH et al. Malformations of cortical development with balloon cells: clinical and radiologic correlates. *Neurology* 2003;60:580-587.
92. GUMBINGER C, ROHSBACH CB, SCHULZE-BONHAGE A et al. Focal cortical dysplasia: A genotype-phenotype type analysis of polymorphisms and mutations in the TSC genes. *Epilepsia* 2009.
93. BECKER AJ, URBACH H, SCHEFFLER B et al. Focal cortical dysplasia of Taylor's balloon cell type: mutational analysis of the TSC1 gene indicates a pathogenic relationship to tuberous sclerosis. *Ann Neurol* 2002;52:29-37.
94. MAJORES M, BLUMCKE I, URBACH H et al. Distinct allelic variants of TSC1 and TSC2 in epilepsy-associated cortical malformations without balloon cells. *J Neuropathol Exp Neurol* 2005;64:629-637.
95. CRINO PB. Molecular pathogenesis of focal cortical dysplasia and hemimegalencephaly. *J Child Neurol* 2005;20:330-336.
96. BAYBIS M, YU J, LEE A et al. mTOR cascade activation distinguishes tubers from focal cortical dysplasia. *Ann Neurol* 2004;56:478-487.
97. MIYATA H, CHIANG AC, VINTERS HV. Insulin signaling pathways in cortical dysplasia and TSC-tubers: tissue microarray analysis. *Ann Neurol* 2004;56:510-519.
98. COTTER D, HONAVAR M, LOVESTONE S et al. Disturbance of Notch-1 and Wnt signalling proteins in neuroglial balloon cells and abnormal large neurons in focal cortical dysplasia in human cortex. *Acta Neuropathol (Berl)* 1999;98:465-472.
99. LOMBROSO CT. Can early postnatal closed head injury induce cortical dysplasia. *Epilepsia* 2000;41:245-253.
100. MARIN-PADILLA M, PARISI JE, ARMSTRONG DL, SARGENT SK, KAPLAN JA. Shaken infant syndrome: developmental neuropathology, progressive cortical dysplasia, and epilepsy. *Acta Neuropathol (Berl)* 2002;103:321-332.
101. DA SILVA AV, REGONDI MC, CAVALHEIRO EA, SPREAFICO R. Disruption of cortical development as a consequence of repetitive pilocarpine-induced status epilepticus in rats. *Epilepsia* 2005;46 Suppl 5:22-30.
102. WONG M. Mechanisms of epileptogenesis in tuberous sclerosis complex and related malformations of cortical development with abnormal glioneuronal proliferation. *Epilepsia* 2008;49:8-21.
103. NAJM IM, TILELLI CQ, OGHIAKIAN R. Pathophysiological mechanisms of focal cortical dysplasia: a critical review of human tissue studies and animal models. *Epilepsia* 2007;48 Suppl 2:21-32.
104. CEPEDA C, ANDRE VM, FLORES-HERNANDEZ J et al. Pediatric cortical dysplasia: correlations between neuroimaging, electrophysiology and location of cytomegalic neurons and balloon cells and glutamate/GABA synaptic circuits. *Dev Neurosci* 2005;27:59-76.
105. ALONSO-NANCLARES L, GARBELLI R, SOLA RG et al. Microanatomy of the dysplastic neocortex from epileptic patients. *Brain* 2005;128:158-173.
106. BOONYAPISIT K, NAJM I, KLEM G et al. Epileptogenicity of focal malformations due to abnormal cortical development: direct electrocorticographic-histopathologic correlations. *Epilepsia* 2003;44:69-76.
107. ANDRE VM, FLORES-HERNANDEZ J, CEPEDA C et al. NMDA receptor alterations in neurons from pediatric cortical dysplasia tissue. *Cereb Cortex* 2004;14:634-646.
108. SPREAFICO R, TASSI L, COLOMBO N et al. Inhibitory circuits in human dysplastic tissue. *Epilepsia* 2000;41 Suppl 6:S168-173.
109. THOM M, HARDING BN, LIN WR, MARTINIAN L, CROSS H, SISODIYA SM. Cajal-Retzius cells, inhibitory interneuronal populations and neuropeptide Y expression in focal cortical dysplasia and microdysgenesis. *Acta Neuropathol (Berl)* 2003;105:561-569.
110. KERFOOT C, VINTERS HV, MATHERN GW. Cerebral cortical dysplasia: giant neurons show potential for increased excitation and axonal plasticity. *Dev Neurosci* 1999;21:260-270.
111. WHITE R, HUA Y, SCHEITHAUER B, LYNCH DR, HENSKE EP, CRINO PB. Selective alterations in glutamate and GABA receptor subunit mRNA expression in dysplastic neurons and giant cells of cortical tubers. *Ann Neurol* 2001;49:67-78.
112. GARBELLI R, MUNARI C, DE BIASI S et al. Taylor's cortical dysplasia: a confocal and ultrastructural immunohistochemical study. *Brain Pathol* 1999;9:445-461.
113. D'ANTUONO M, LOUVEL J, KOHLING R et al. GABAA receptor-dependent synchronization leads to ictogenesis in the human dysplastic cortex. *Brain* 2004;127:1626-1640.
114. MEENCKE HJ, JANZ D. Neuropathological findings in primary generalized epilepsy: a study of eight cases. *Epilepsia* 1984;25:8-21.
115. NORDBORG C, ERIKSSON S, RYDENHAG B, UVEBRANT P, MALMGREN K. Microdysgenesis in surgical specimens from patients with epilepsy: occurrence and clinical correlations. *J Neurol Neurosurg Psychiatry* 1999;67:521-524.
116. KASPER BS, STEFAN H, BUCHFELDER M, PAULUS W. Temporal lobe microdysgenesis in epilepsy versus control brains. *J Neuropathol Exp Neurol* 1999;58:22-28.
117. GARBELLI R, FRASSONI C, FERRARIO A, TASSI L, BRAMERIO M, SPREAFICO R. Cajal-Retzius cell density as marker of type of focal cortical dysplasia. *Neuroreport* 2001;12:2767-2771.

118. THOM M, SISODIYA S, HARKNESS W, SCARAVILLI F. Microdysgenesis in temporal lobe epilepsy. A quantitative and immunohistochemical study of white matter neurones. *Brain* 2001;124:2299-2309.
119. THOM M, MARTINIAN L, SEN A, CROSS JH, HARDING BN, SISODIYA SM. Cortical neuronal densities and lamination in focal cortical dysplasia. *Acta Neuropathol (Berl)* 2005;110:383-392.
120. HARDIMAN O, BURKE T, PHILLIPS J et al. Microdysgenesis in resected temporal neocortex: incidence and clinical significance in focal epilepsy. *Neurology* 1988;38:1041-1047.
121. EMERY JA, ROPER SN, ROJIANI AM. White matter neuronal heterotopia in temporal lobe epilepsy: a morphometric and immunohistochemical study. *J Neuropathol Exp Neurol* 1997;56:1276-1282.
122. BOTHWELL S, MEREDITH GE, PHILLIPS J et al. Neuronal hypertrophy in the neocortex of patients with temporal lobe epilepsy. *J Neurosci* 2001;21:4789-4800.
123. HILDEBRANDT M, PIEPER T, WINKLER P, KOLODZIEJCZYK D, HOLTHAUSEN H, BLUMCKE I. Neuropathological spectrum of cortical dysplasia in children with severe focal epilepsies. *Acta Neuropathol* 2005;110:1-11.
124. ERIKSSON SH, FREE SL, THOM M, MARTINIAN L, SISODIYA SM. Methodological aspects of 3D and automated 2D analyses of white matter neuronal density in temporal lobe epilepsy. *Neuropathol Appl Neurobiol* 2006;32:260-270.
125. PASQUIER B, PEOC HM, FABRE-BOCQUENTIN B et al. Surgical pathology of drug-resistant partial epilepsy. A 10-year-experience with a series of 327 consecutive resections. *Epileptic Disord* 2002;4:99-119.
126. BLUMCKE I, WIESTLER OD. Gangliogliomas: an intriguing tumor entity associated with focal epilepsies. *J Neuropathol Exp Neurol* 2002;61:575-584.
127. BLUMCKE I, LUYKEN C, URBACH H, SCHRAMM J, WIESTLER OD. An isomorphic subtype of long-term epilepsy-associated astrocytomas associated with benign prognosis. *Acta Neuropathol (Berl)* 2004;107:381-388.
128. WANG M, TIHAN T, ROJIANI AM et al. Monomorphous angiocentric glioma: a distinctive epileptogenic neoplasm with features of infiltrating astrocytoma and ependymoma. *J Neuropathol Exp Neurol* 2005;64:875-881.
129. CENACCHI G, GIANGASPERO F. Emerging tumor entities and variants of CNS neoplasms. *J Neuropathol Exp Neurol* 2004;63:185-192.
130. EDGAR MA, ROSENBLUM MK. Mixed glioneuronal tumors: recently described entities. *Arch Pathol Lab Med* 2007;131:228-233.
131. DAUMAS-DUPORT C. Dysembryoplastic neuroepithelial tumours. *Brain Pathol* 1993;3:283-295.
132. HONAVAR M, JANOTA I, POLKEY CE. Histological heterogeneity of dysembryoplastic neuroepithelial tumour: identification and differential diagnosis in a series of 74 cases. *Histopathology* 1999;34:342-356.
133. SHARMA MC, JAIN D, GUPTA A et al. Dysembryoplastic neuroepithelial tumor: a clinicopathological study of 32 cases. *Neurosurg Rev* 2009;32:161-169; discussion 169-170.
134. LUYKEN C, BLUMCKE I, FIMMERS R et al. The spectrum of long-term epilepsy-associated tumors: long-term seizure and tumor outcome and neurosurgical aspects. *Epilepsia* 2003;44:822-830.
135. DUGGAL N, TAYLOR R, ZOU GY, HAMMOND RR. Dysembryoplastic neuroepithelial tumours: clinical, proliferative and apoptotic features. *J Clin Pathol* 2008;61:127-131.
136. GONZALES M, DALE S, SUSMAN M et al. Dysembryoplastic neuroepithelial tumor (DNT)-like oligodendrogliomas or Dnts evolving into oligodendrogliomas: two illustrative cases. *Neuropathology* 2007;27:324-330.
137. RAY WZ, BLACKBURN SL, CASAVILCA-ZAMBRANO S et al. Clinicopathologic features of recurrent dysembryoplastic neuroepithelial tumor and rare malignant transformation: a report of 5 cases and review of the literature. *J Neurooncol* 2009;94:283-292.
138. HIROSE T, SCHEITHAUER BW. Mixed dysembryoplastic neuroepithelial tumor and ganglioglioma. *Acta Neuropathol (Berl)* 1998;95:649-654.
139. SHIMBO Y, TAKAHASHI H, HAYANO M, KUMAGAI T, KAMEYAMA S. Temporal lobe lesion demonstrating features of dysembryoplastic neuroepithelial tumor and ganglioglioma: a transitional form? *Clin Neuropathol* 1997;16:65-68.
140. BECKER AJ, LOBACH M, KLEIN H et al. Mutational analysis of TSC1 and TSC2 genes in gangliogliomas. *Neuropathol Appl Neurobiol* 2001;27:105-114.
141. KAM R, CHEN J, BLUMCKE I et al. The reelin pathway components disabled-1 and p35 in gangliogliomas--a mutation and expression analysis. *Neuropathol Appl Neurobiol* 2004;30:225-232.
142. LUYKEN C, BLUMCKE I, FIMMERS R, URBACH H, WIESTLER OD, SCHRAMM J. Supratentorial gangliogliomas: histopathologic grading and tumor recurrence in 184 patients with a median follow-up of 8 years. *Cancer* 2004;101:146-155.
143. ARONICA E, YANKAYA B, JANSEN GH et al. Ionotropic and metabotropic glutamate receptor protein expression in glioneuronal tumours from patients with intractable epilepsy. *Neuropathol Appl Neurobiol* 2001;27:223-237.
144. WOLF HK, BUSLEI R, BLUMCKE I, WIESTLER OD, PIETSCH T. Neural antigens in oligodendrogliomas and dysembryoplastic neuroepithelial tumors. *Acta Neuropathol (Berl)* 1997;94:436-443.
145. BLUMCKE I, MULLER S, BUSLEI R, RIEDERER BM, WIESTLER OD. Microtubule-associated protein-2 immunoreactivity: a useful tool in the differential diagnosis of low-grade neuroepithelial tumors. *Acta Neuropathol (Berl)* 2004;108:89-96.
146. BLUMCKE I, BECKER AJ, NORMANN S et al. Distinct expression pattern of microtubule-associated protein-2 in human oligodendrogliomas and glial precursor cells. *J Neuropathol Exp Neurol* 2001;60:984-993.

147. FASSUNKE J, MAJORES M, ULLMANN C et al. In situ-RT and immunolaser microdissection for mRNA analysis of individual cells isolated from epilepsy-associated glioneuronal tumors. *Lab Invest* 2004;84:1520-1525.
148. DAUMAS-DUPORT C, VARLET P, BACHA S, BEUVON F, CERVERA-PIEROT P, CHODKIEWICZ JP. Dysembryoplastic neuroepithelial tumors: nonspecific histological forms -- a study of 40 cases. *J Neurooncol* 1999;41:267-280.
149. BLUMCKE I, GIENCKE K, WARDELMANN E et al. The CD34 epitope is expressed in neoplastic and malformative lesions associated with chronic, focal epilepsies. *Acta Neuropathol (Berl)* 1999;97:481-490.
150. ARONICA E, GORTER JA, JANSEN GH, LEENSTRA S, YANKAYA B, TROOST D. Expression of connexin 43 and connexin 32 gap-junction proteins in epilepsy-associated brain tumors and in the perilesional epileptic cortex. *Acta Neuropathol (Berl)* 2001;101:449-459.
151. ZHAO J, WANG S, LI J, QI W, SUI D, ZHAO Y. Clinical characteristics and surgical results of patients with cerebral arteriovenous malformations. *Surg Neurol* 2005;63:156-161; discussion 161.
152. MORAN NF, FISH DR, KITCHEN N, SHORVON S, KENDALL BE, STEVENS JM. Supratentorial cavernous haemangiomas and epilepsy: a review of the literature and case series. *J Neurol Neurosurg Psychiatry* 1999;66:561-568.
153. STEFAN H, HAMMEN T. Cavernous haemangiomas, epilepsy and treatment strategies. *Acta Neurol Scand* 2004;110:393-397.
154. VOLK EE, PRAYSON RA. Hamartomas in the setting of chronic epilepsy: a clinicopathologic study of 13 cases. *Hum Pathol* 1997;28:227-232.
155. DIEHL B, PRAYSON R, NAJM I, RUGGIERI P. Hamartomas and epilepsy: clinical and imaging characteristics. *Seizure* 2003;12:307-311.
156. GOMEZ-ANSON B, THOM M, MORAN N, STEVENS J, SCARAVILLI F. Imaging and radiological-pathological correlation in histologically proven cases of focal cortical dysplasia and other glial and neuronogial malformative lesions in adults. *Neuroradiology* 2000;42:157-167.
157. KERRIGAN JF, NG YT, CHUNG S, REKATE HL. The hypothalamic hamartoma: a model of subcortical epileptogenesis and encephalopathy. *Semin Pediatr Neurol* 2005;12:119-131.
158. BALESTRI P, VIVARELLI R, GROSSO S et al. Malformations of cortical development in neurofibromatosis type 1. *Neurology* 2003;61:1799-1801.
159. VIVARELLI R, GROSSO S, CALABRESE F et al. Epilepsy in neurofibromatosis 1. *J Child Neurol* 2003;18:338-342.
160. PERRY A, KURTKAYA-YAPICIER O, SCHEITHAUER BW et al. Insights into meningoangiomas with and without meningioma: a clinicopathologic and genetic series of 24 cases with review of the literature. *Brain Pathol* 2005;15:55-65.
161. WIEBE S, MUNOZ DG, SMITH S, LEE DH. Meningioangiomas. A comprehensive analysis of clinical and laboratory features. *Brain* 1999;122 (Pt 4):709-726.
162. PARDO CA, VINING EP, GUO L, SKOLASKY RL, CARSON BS, FREEMAN JM. The pathology of Rasmussen syndrome: stages of cortical involvement and neuropathological studies in 45 hemispherectomies. *Epilepsia* 2004;45:516-526.
163. BIEN CG, BAUER J, DECKWERTH TL et al. Destruction of neurons by cytotoxic T cells: a new pathogenic mechanism in Rasmussen's encephalitis. *Ann Neurol* 2002;51:311-318.
164. BIEN CG, GRANATA T, ANTOZZI C et al. Pathogenesis, diagnosis and treatment of Rasmussen encephalitis: a European consensus statement. *Brain* 2005;128:454-471.
165. TOBIAS SM, ROBITAILLE Y, HICKEY WF, RHODES CH, NORDGREN R, ANDERMANN F. Bilateral Rasmussen encephalitis: postmortem documentation in a five-year-old. *Epilepsia* 2003;44:127-130.