

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 effects of any primary, underlying neurological disease process that has led to increased seizure susceptibility. Although there is evidence to support detrimental effects of seizures on brain histology, this is not inevitable^{1,2}. Prolonged seizures may result in neuronal death by apoptotic (programmed or 'active') or necrotic pathways, as well as gliosis and microglial activation. Furthermore, injurious effects of seizures should be evaluated not only by histological changes, but at the subcellular, synaptic and molecular level. Neuropathological alterations may be adaptive and reversible while others are permanent.

POST MORTEM EXAMINATIONS IN EPILEPSY

Post mortems carried out in patients with epilepsy include both Coroner's examinations (particularly in unexpected and accidental deaths) and hospital post mortems, requested by the clinician with consent of the family. Epilepsy-related deaths are those where seizures may have directly contributed to the cause of death. In the examination of a brain from a patient with epilepsy, the neuropathologist addresses three main 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? iii) is there any direct contribution of epilepsy to the cause of death. Regional tissue sampling is guided by any macroscopic abnormality or from localising clinical, electrophysiological and/or neuroimaging data. For secondary changes from seizures, regions of brain most vulnerable include the hippocampus, neocortex, thalamus, amygdala and cerebellum.

Epilepsy-related deaths

Status epilepticus: In status epilepticus, normal inhibitory mechanisms fail and epileptic activity becomes self-sustaining. Identified pathomechanisms include endocytosis and down-regulation of GABA_A receptors³ along with recruitment of AMPA and NMDA receptors, with an overall pro-convulsant effect. Over a longer time course, depletion of inhibitory peptides dynorphin, galanin, somatostatin and neuropeptide Y occurs, while pro-convulsants including substance P increase, acting to self-sustain seizures⁴. Neuropathological findings reported following fatal cases of status epilepticus (SE) include neuronal loss or injury in hippocampal CA1, CA3 and the hilus (the dentate granule cells may be spared⁵), amygdala (corticomedial and basolateral nuclei), neocortex (mid-cortical layers), the entorhinal cortex, Purkinje cell layer of the cerebellum⁶, mamillary bodies⁷, the dorsal medial nuclei of the thalamus⁸ and basal ganglia⁵. The neuronal damage may be predominantly unilateral in some cases⁸ and with histories of prolonged hemi-convulsions cerebral hemiatrophy can eventually occur with striking unilateral laminar necrosis of the second to fourth cortical layers⁹. Neuronal loss, however, is not an inevitable consequence of status epilepticus.

Traumatic brain injury: Traumatic lesions may be a cause of epilepsy but are also a consequence. Patients with generalised seizures have a higher risk of minor and severe cerebral injuries, including cerebral haemorrhage and contusions; the risk is related to seizure frequency, type and control^{10,11}. Post mortem examinations may identify old cystic cortical contusions, particularly in the fronto-temporal regions, present in 30% in one series, and may increase vulnerability to age-accelerated neurofibrillary tangle pathology¹².



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

Neurodegeneration. Epilepsy may form part of the clinical manifestations of various neurodegenerative diseases and other common neurological conditions such as strokes and inflammatory conditions including MS¹³. Patients with Alzheimer's disease are at increased risk for developing epilepsy; the underlying epileptogenic mechanism is unknown, but the toxic effects of amyloid- β on synaptic transmission have been considered¹⁴.

Sudden death in epilepsy

Neuropathology: Post mortem examination is mandatory in sudden unexpected death in epilepsy (SUDEP), primarily to exclude an anatomical (e.g. cardiac) or other cause of death. 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 absent¹⁵. It is 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^{16,17}. 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 malformations¹⁶. There is no accurate data regarding the relative risk or association of any of these specific pathological lesions for SUDEP. Some lesions, including acquired 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 to investigate any unsuspected pathology, e.g. meningo-encephalitis.

It is not possible or necessary for a neuropathologist to perform all autopsies on patients with epilepsy. Ideally, a specialist neuropathologist should be involved in the interpretation of the histological brain 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 practice 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, pathology is more likely to be overlooked¹⁷.

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 SUDEP¹⁸. In subsequent studies however, no difference in heart mass compared to non-SUDEP controls was noted when corrected for body mass^{19,20}. Extensive sampling of the myocardium in SUDEP 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 fibres²⁰. 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 controls²¹. Cardiac fibrosis has not however been reported in all post mortem SUDEP series¹⁸. 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 cases²², may be a more cautious approach. Pulmonary oedema has been reported in 50–90% of SUDEP cases^{15,16,18}. Lung weights in SUDEP cases did not differ from non-SUDEP cases¹⁹ in another 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 indicated²³. Molecular/genetic testing for channelopathies in SUDEP is likely to become more routine^{24,25}, for example SUDEP is more frequent in patients with Dravet syndrome and SCN1a mutations²⁶.

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. SUDEP can be further classified as ‘definite’ or ‘possible’ (cases where there is a competing cause of death at post mortem) or ‘probable’ where the autopsy data is incomplete²⁷. 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 by the Royal College of Pathologists. To make progress in understanding what causes SUDEP, which is one step towards its prevention, a ‘global action’ is required²⁸, with team work between multidisciplinary professionals, including neuropathologists.

SURGICAL NEUROPATHOLOGY AND FOCAL EPILEPSIES

Epilepsy surgery has been established as an effective treatment option in pharmacoresistant focal epilepsies^{29,30}. Following continued advances in imaging technology, structural lesions are increasingly recognised in patients with chronic focal seizures. Electrophysiological evaluation, for example surface or invasive EEG recordings, may further define the epileptogenic area prior to tissue resection.

In many centres surgical tissues are sent immediately, fresh to the laboratory, to allow the opportunity for appropriate freezing, fixing and banking of tissue samples for both diagnostic and research purposes³¹. Common specimens include lobectomies (temporal or frontal), hippocampectomies or lesionectomies. Common pathologies recognised are presented in the Table below; the incidence may vary according to the age group included, with malformations more common in paediatric cohorts. In some resections (particularly if ‘MRI-negative’) no-specific lesion is identified and gliosis or microgliosis may be the only histology finding. In other cases, a double or dual pathology is found.

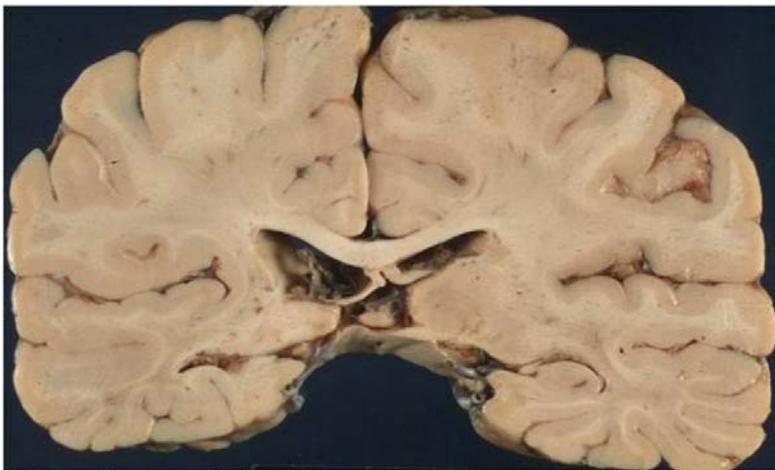


Surgical temporal lobectomy specimen received fresh in laboratory (and hippocampus below) allows correct orientation and sampling to optimise both diagnostic and research work.

Year	Region	Age of patients Mean/range	Number of cases	Focal dysplasias	Vascular malformations	Tumours	Hippocampal sclerosis	Atrophic lesion (Old infarcts/traumatic lesions/ scars)	Inflammatory pathology	Gliosis or no pathology	Dual pathology cases (HS+lesion)	Other
2002	Grenoble	All ages	327	18%		28.70%	25.90%	5%	1.2%	15.50%	5%	
2008	Multi-centre	Paediatric	413	42.4%	1.5%	19.1%	6.50%	9.9%	2.7%	6.30%	13.8%	3.6%
2009	German epilepsy register	All ages	4512	12.70%	6%	27.30%	35.20%	5.20%	1.60%	6.80%	5%	
2010	Beijing	All ages	435	52.90%		11.70%	17%	22.80%				

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) but can be seen in other epilepsy syndromes as well as in ageing without epilepsy³². The neuropathological features have been recognised for over a century (for historical review see Thom 2009³³) but its cause, and in turn how it causes epilepsy, is still the focus of 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 of the hippocampal formation (see Figure below). 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 previously referred to as 'classical' hippocampal sclerosis. 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 preserved, although in 40–50% of cases shows dispersion or looser packing of the neurones. 'End folium sclerosis' describes a pattern of neuronal damage confined to the hilar and CA4 pyramidal cells in a small number of patients. In other cases neuronal loss may appear restricted to CA1 region.

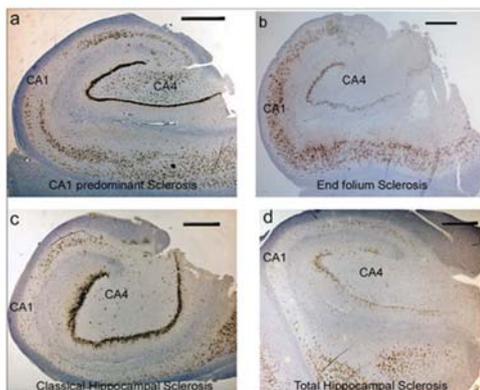
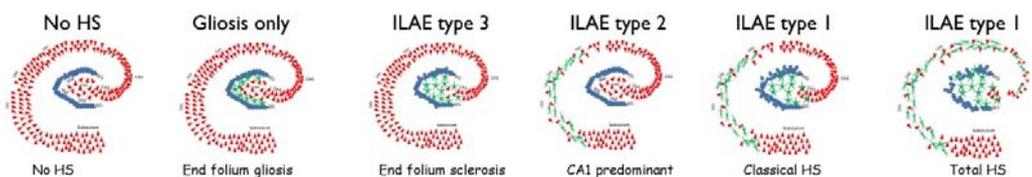
There is no single explanation for the regional selectivity of pyramidal cell loss between subfields; excitatory pathways and networks, altered inhibitory input, and the effectiveness and variability of endogenous neuroprotective mechanisms are likely to be involved.

Several schemes have been previously used for the sub-classification and quantitation of neuronal loss in hippocampal sclerosis as the Wyler system and many quantitative evaluations carried out (see review³⁴). The ILAE system, based on the semi-quantitative analysis of subfields, was introduced in 2013, is reproducible between centres and integrates previous schemes (see Table below) and has been shown to provide prognostic information in terms of outcome³⁵.

Clinicopathological correlations also show correlation with ILAE subtype and pre-operative memory³⁶. Different HS subtypes may be detectable with high field MRI³⁷.

Subfield	Severity of neuronal loss			
	0-2	0-1	0-2	0-1
Dentate gyrus	0-2	0-1	0-2	0-1
CA4	2	0-1	1-2	0
CA3	0-2	0-1	0-1	0
CA2	0-2	0-1	0-1	0
CA1	2	1-2	0-1	0
Subiculum	0	0	0	0
Classification	HS ILAE type 1	HS ILAE type 2	HS ILAE type 3	No HS
Previous terminologies	Classical/Total/ Severe HS	CA1 predominant HS	End folium sclerosis or CA4 predominant	No HS

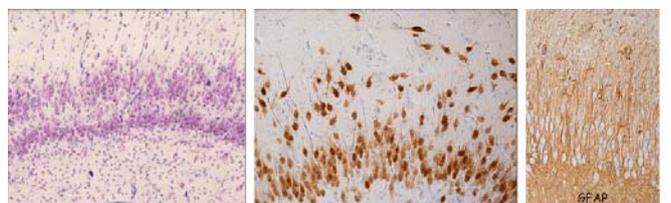
Table. ILAE Classification for hippocampal sclerosis (HS) 2013. Semi-quantitative microscopic examination based on formalin-fixed, paraffin-embedded surgical specimen (4–7 µm section thickness), haematoxylin and eosin staining, cresyl-violet combined with Luxol-fast blue staining (CV/LFB; Figure 1), GFAP immunohistochemistry (Figure 3) and NeuN immunohistochemistry (recommended; Figure 4). The scoring system refers to neuronal cell loss (NeuN staining) and is defined for Subiculum and CA1 to CA4 as following: 0 = No obvious neuronal loss or moderate astrogliosis only; 1 = Neuronal loss and gliosis (GFAP) is moderate or patchy is patchy more or less than moderate; 2 = severe neuronal loss (majority of neurons lost) and fibrillary astrogliosis. Scores for the Dentate Gyrus (DG): granule cell layer is normal (score = 0), dispersed (score 1; can be focal) or shows severe granule cell loss (score 2; can be focal) (Blumcke et al, 2013). The ILAE subtypes are shown in the diagram below; pyramidal neurones of hippocampal subfields and subiculum are shown in red, granule cells in blue and astrogliosis in green (taken from Thom 2014³⁸).



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

Associated findings with hippocampal sclerosis

(i) **Dispersion of granule cells** into the molecular layer, associated with hippocampal sclerosis was first described by Houser³⁹. This phenomenon appears peculiar to seizure-induced hippocampal damage and is encountered in 40–50% of HS cases in surgical series^{40–43}. 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^{41,44}. 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). 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



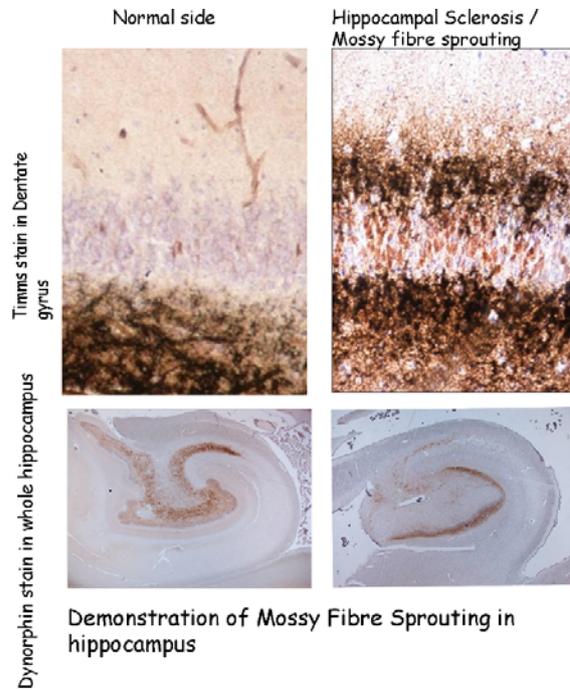
Patterns of Granule cell dispersion : A bilaminar pattern on the left, single dispersed neurones as seen with NeuN (centre) and the accompanying gliosis as seen with GFAP on right.

cell layer thicker than 10 cells⁴² or 120 μm has been proposed. In many cases the thickness may in fact reach 200 μm or greater⁴⁵, compared to mean control widths of around 100 μm . There is a sub-classification system for types of dispersion⁴⁶, although no correlation with outcome following surgery has been demonstrated in any study^{46,47}. There is an association between GCD and early onset of seizures.

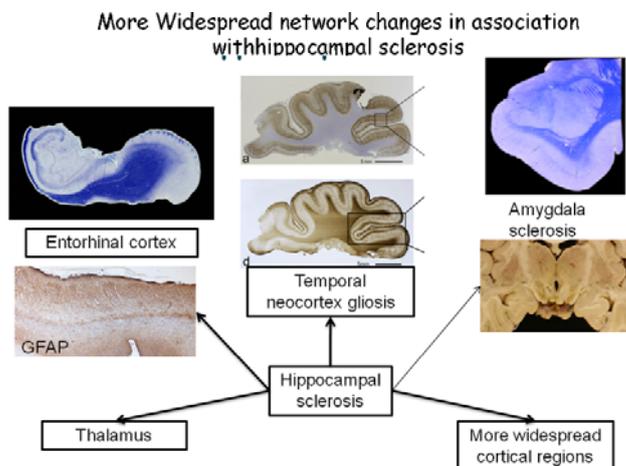
The functional significance as well as the mechanism for granule cell dispersion is unclear. Dispersion of granule cells has also been demonstrated in experimental models of TLE. For example, granule cell dispersion is observed in the kainate models, first appearing at about four days following seizures, increasing over eight weeks and persisting for at least six months⁴⁸. 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 gyrus⁴⁴. It may relate to local reelin deficiency^{49,50}, altered rates of neurogenesis stimulated by seizures or differential expression of miRNA⁵¹.

(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 sprouting (MFS). The majority (over 90%) of these sprouted mossy fibres appear to make synaptic contact (excitatory asymmetric synapses)⁵² with apical dendrites and spines of granule cells in the inner molecular layer, and a smaller proportion with inhibitory interneurons⁵². 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 of, rather than directly provoking, seizures⁵³. Mossy fibre sprouting is best visualised (in both experimental and human tissue) with Timm silver method (as mossy fibre boutons contain high levels of zinc). 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 as well as with labelling for zinc transporter 3 (ZnT3)⁵⁴.



(iii) **Widespread changes in association with hippocampal sclerosis in TLE.** Pathology may extend beyond the hippocampus, involving adjacent structures (as well as regions connected with the hippocampus). These additional pathologies (usually neuronal loss, gliosis) may be relevant to seizure onset (and epileptogenesis), seizure networks, associated co-morbid features including neuropsychological impairment and poor outcome following surgical treatment.



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 ventromedial aspects are more severely affected⁵⁵. In addition, the basal nuclei, particularly the parvocellular division, may be involved⁵⁶. 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 diagnosis⁴². As a result, reports on the incidence of amygdala sclerosis varies between institutions from 35–76%⁵⁵. Greater amygdala neuronal loss has been shown in patients with hippocampal sclerosis, although amygdala sclerosis has also been reported in isolation⁴². Amygdala enlargement has been reported in TLE but the underlying pathology substrate is variable and includes tumours and malformation.

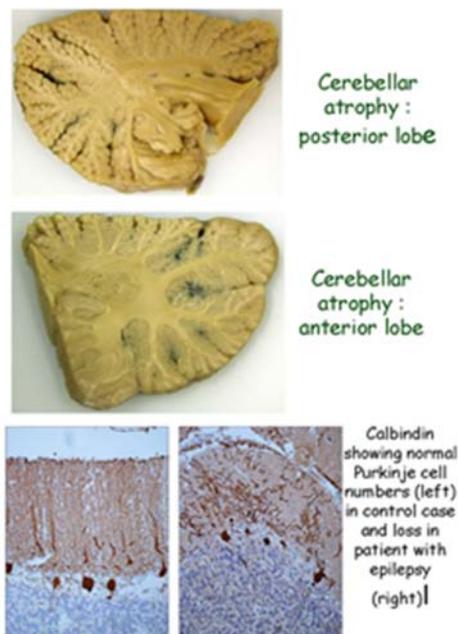
Entorhinal cortex (EC) in HS: There is also evidence that characteristic pathological changes are present in the entorhinal cortex (EC) in patients with hippocampal sclerosis and that this region may have importance in the initiation of mesial temporal lobe seizures or development of HS. The EC at the 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. Neurones from superficial layers (mainly layers II and III) send glutamatergic afferents, via the perforant pathway, to the dentate granule cells and CA1 neurones; subicular and CA1 pyramidal neurones have feedback connections to the deeper layers of the EC. Neuroimaging studies have reported volume reduction of parahippocampal gyral structures in TLE, mainly ipsilateral to the seizures^[57] and abnormal epileptiform activity has been recorded in the EC region⁵⁸, which may sustain seizures. In pathological studies of animal models of TLE, and post-status, selective vulnerability of layer III neurones was shown^{59,60}. 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^{55,61}.

Neocortex: In the neocortex, loss of neurones, when present, is most apparent in mid-cortical layers with associated gliosis. Subcortical white matter gliosis, 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 IIIa (see section below for dysplasia) (for review of temporal lobe sclerosis see Thom et al 2009⁶²). Quantitative post mortem studies of patients with hippocampal sclerosis also support more widespread neocortical pathology⁶³.

Thalamus: Volume reduction of the thalamus has been observed with MRI studies⁶⁴, as well as in post mortem⁶⁵ and experimental studies⁶⁶ of epilepsy, and is more closely linked with hippocampal and amygdala atrophy and TLE. Neuronal loss and gliosis may be widespread but with some evidence for greater involvement of dorsomedial nuclei⁶⁷. Thalamic atrophy is more often unilateral and associated atrophy of the fornix and mamillary bodies may also be seen. Proposed pathomechanisms 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 identified⁶, present in 25% of cases in one post mortem series⁶⁵. 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

patients⁶⁸. 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 MRI⁶⁹. Neuropathological findings at post mortem may disclose preferential symmetrical atrophy of the anterior lobes or the more common pattern involving the posterior lobes⁷⁰. 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.



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 epilepticus^[71]), antiepileptic drug (AED) toxicity (in particular phenytoin), hypoxic-ischaemic injury, trauma as a result of seizures⁷⁰ 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 administration⁷⁰, and experimental toxicity to Purkinje cells and granule cells has been shown^{72,73}. There is no consistent relationship between seizure frequency and duration and the degree of atrophy to argue for either process^{68–71}. 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 occur⁷⁴. Seizure control post temporal lobectomy was shown not to be influenced by the presence of cerebellar atrophy in one⁷⁵ but not another⁷⁶ study.

CORTICAL MALFORMATIONS IN SURGICAL RESECTIONS FOR EPILEPSY

Focal cortical dysplasias – nomenclature

Cortical dysplasia refers to a subtype of malformations due to abnormal cortical development (MCD) where the abnormality is strictly or largely intracortical⁷⁷. 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 Palmini⁷⁷, 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)⁷⁸. The rationale was 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.

Incidence of FCD: Based on published series between 2005 and 2011 the relative incidence of reported isolated FCD types is type Ia (46%), type Ib (19%), type IIa (15%) and type IIb (39%);

Table. ILAE 2011 Classification for cortical dysplasia.

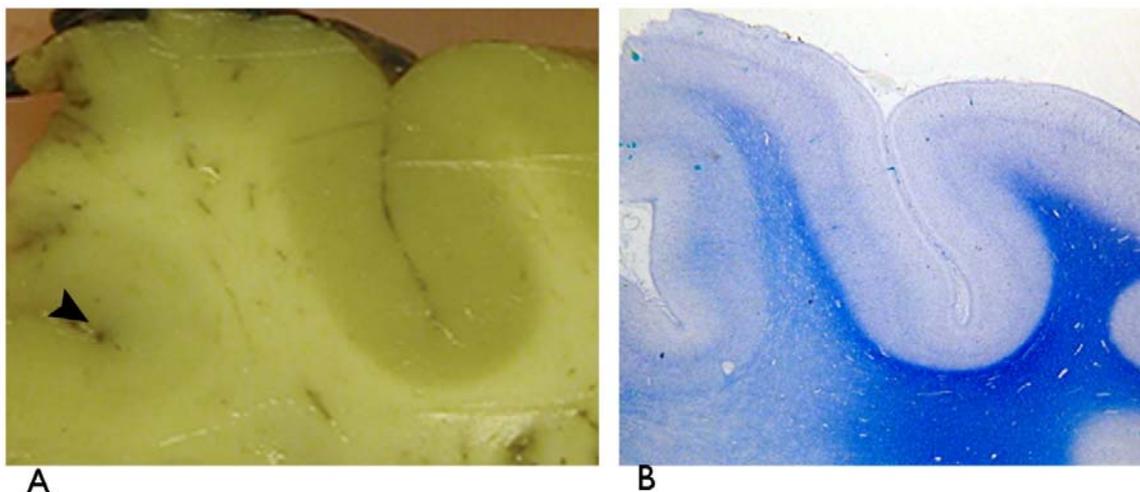
FCD Type I (isolated)	Focal Cortical Dysplasia with abnormal radial cortical lamination (FCD Ia)	Focal Cortical Dysplasia with abnormal tangential cortical lamination (FCD Ib)	Focal Cortical Dysplasia with abnormal radial and tangential cortical lamination (FCD Ic)	
FCD Type II (isolated)	Focal Cortical Dysplasia with dysmorphic neurones (FCD IIa)		Focal Cortical Dysplasia with dysmorphic neurones and balloon cells (FCD IIb)	
FCD Type III (associated with principal lesion)	Focal Cortical Dysplasia in the temporal lobe associated with Hippocampal sclerosis (FCD IIIa)	Focal Cortical Dysplasia adjacent to a glial or glo-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)

the outcome following surgery is that 45%, 49%, 65% and 84% of patients with these subtypes, respectively, become seizure free.

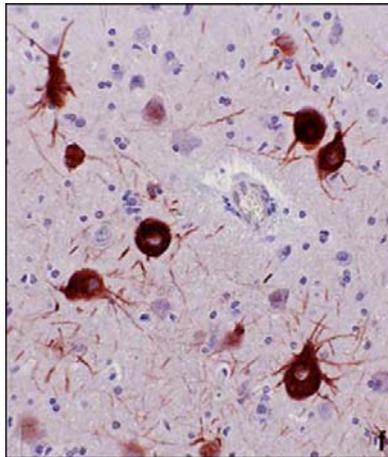
FCD Type II Neuropathology: The macroscopic appearances from an FCD lesion may show a region of apparent thickening of the grey matter, blurring of the grey-white boundary and the tissue may appear firmer. The overall size of these lesions varies and can be up to several centimetres broad involving both sulci and gyri; occasionally discontinuous regions of dysplasia are noted and in young individuals extensive involvement of the hemisphere may be 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 (dyslaminar) 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 neurones compared to normal cortex may be present in FCD type II whereas in FCD I, particularly in childhood epilepsy, an exaggeration of columnar arrangement has been reported^{79,80}. Care should obviously be taken with the interpretation of any cytoarchitectural ‘abnormality’ in regard to normal cortical regional variations. Abnormalities of the cortical myelo-architecture may be striking features in FCD and myelin rarefaction in sub-cortical white matter is a common finding.

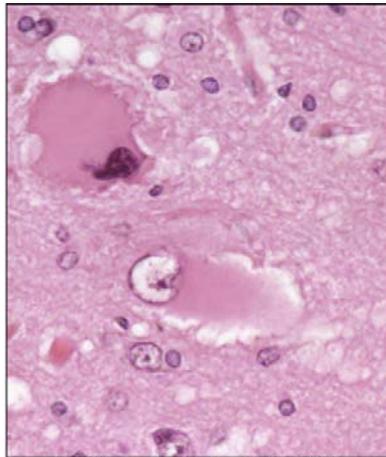
Profoundly abnormal cortical cell types are present in FCD and define the subtypes. **Dysmorphic neurones** have abnormal size, orientation, dendritic processes and cytoskeletal structure. In Cresyl violet stained sections Nissl appears abnormally clumped and eccentric thickening of nuclear membranes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



Macroscopic features of FCD type II with the region of dysplasia (arrowed) showing poor definition between the white and grey matter. In LFB myelin sections this corresponds to reduction of myelinated fibres in the white matter (blue stain) and increased myelination in the cortex.



Dysmorphic neurones in FCD II
neurofilament positive



Balloon cells in FCDIIB

from slight rotation to complete inversion in relation to the pial surface⁸¹. 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). Multinucleate

or giant cell forms are frequent. 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 cells⁸¹. **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’⁸². Cytomegalic GABAergic neurones have been identified as a component of FCD⁸³. Although these abnormal cell types are instantly recognisable in routine sections, immunohistochemistry allows further characterisation and classification. 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. Whether this is a primary or secondary effect is not known. In addition, in many abnormal cell types in FCD there is aberrant expression of developmentally-regulated proteins. Balloon cells also show variable expression of nestin, vimentin, GFAP, GFAP delta, doublecortin, neurofilaments, and MAP1B, an immature MAP isoform⁸⁴. 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^{85,86}. Co-expression of neuronal and glial markers by abnormal cell types has been shown^{84,87}, confirming aberrant glial-neuronal differentiation and this observation, together with the expression of immature proteins, lends support to a common mal-developmental origin.

Current theories propose FCD represents a developmental abnormality with arrested neuronal migration and differentiation. Dysregulation of cell cycle proteins in balloon cells in FCD has been shown⁸⁸, which may represent a pathological progenitor cell type⁸⁹. However overall reductions 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 superimposed neuronal loss. FCD is a sporadic disorder. There are no known family cases of FCD. The pathology of FCDIIB bears similarities to hemimegalencephaly and tuberous sclerosis, and dysregulation of the insulin signalling mTOR/p70SK-S6 pathway (which influences cell size and proliferation) has been shown in FCD⁹⁰. The functional importance of

mTOR pathway has been demonstrated through prevention of seizures in conditional knockout TSC1 animal models that show some of the pathological features of TSC, by treatment with rapamycin, an mTOR inhibitor. Early clinical trials of mTOR inhibitors in the treatment of TSC are also encouraging, with reduction in lesion size. A recent study detected human papilloma virus in balloon cells in FCD IIB but not in TS lesions⁹¹ but this finding has not been replicated⁹². Mutations in DEPDC5, PTEN and PIK3CA have been reported in FCD^{93,94}.

Epilepsy is clearly associated with FCD lesions. Single cell recordings from dysplastic neurons have demonstrated abnormal intrinsic membrane properties and ion channel functions⁸¹ although no spontaneous epileptiform depolarisations have been shown, suggesting they are unlikely to operate as ‘pacemaker’ neurons⁹⁵. Immature balloon cells do not display spontaneous synaptic current or action potentials⁸¹ and lack synaptic contacts suggesting they are inert bystanders. Studies of glutamate transporters propose that balloon cells might exhibit a protective effect against local ictal activity, through increased glutamate clearance mechanisms⁸⁶. Altered assembly of NMDA and AMPA receptors has been shown in neurones in FCD and alteration of the GABAergic system is also evident in FCD II with reduction of interneurons and cytomegalic cells reported⁹⁵. Abnormal expression of cationic-chloride co-transporters that regulate intracellular chloride and influence the capacity of GABA to generate hyperpolarising inhibitory potentials, have been shown in FCD. Studies of gap junction proteins, including Cx43 have also shown abnormal aggregates around balloon cells in FCD IIB, which could be of functional significance in the formation of abnormal local networks. Additionally, increased and altered distribution of Aquaporin 4 in relation to the dysplastic neurones has been shown in FCD II which may influence local fluid homeostasis and modify neuronal function. Finally, increasing interest has been directed at the contribution of pro-inflammatory mechanisms in FCD II in epileptogenesis.

Mild malformations of cortical development (MCD)

Mild MCD in epilepsy encompass more subtle cortical abnormalities previously referred to as microdysgenesis or ‘architectural dysplasias’. Mild MCD are divided into two categories: Type I with ectopic neurones placed in or adjacent to layer I; and type II with microscopic single neuronal heterotopia outside layer I including the white matter. The precise aetiology of these subtle abnormalities, their relevance in epilepsy and significance in terms of predicting outcome following surgery remains less certain, compared to for example FCD IIB⁹⁶.

FCD type III

FCD type IIIa refers to a variety of alterations in cortical architectural organisation observed in patients with HS. The aetiology and pathogenesis of FCD Type IIIa remains to be determined, but is likely an associated process to MTS. One common type of FCD IIIa, also called ***temporal lobe sclerosis***, clusters of neurones are observed in the outer part of layer II⁶². It is accompanied by severe neuronal cell loss in layer II and III with associated laminar gliosis (see Figure below). It is observed in around 10% of patients with HS in the context of TLE and may have been acquired early in life following a precipitating injury, such as a febrile seizure. Horizontal bundles of myelinated axons can be observed to a variable degree in all cases. FCD type IIIb refers to dysplasias observed adjacent to glioneuronal tumours and FCD IIIc and FCD IIIId to those observed in the context of underlying vascular malformation and early vascular/inflammatory disease processes respectively.

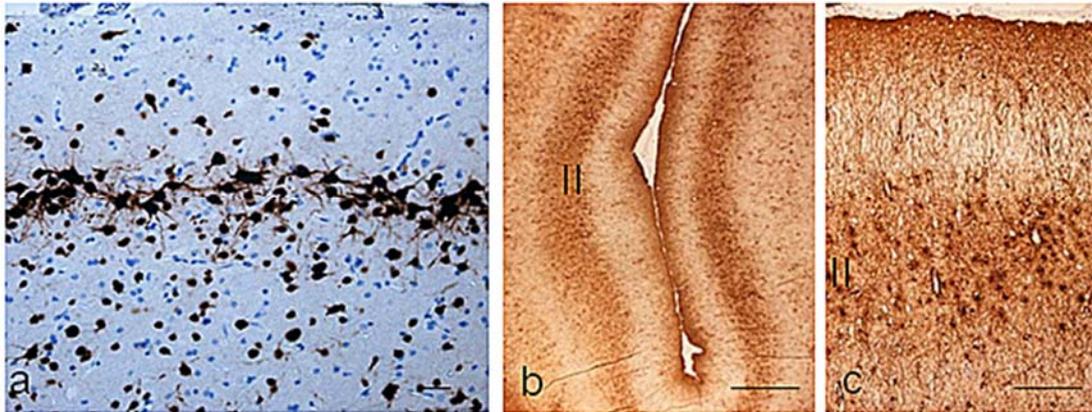


Figure. FCDIIIb. Temporal neocortex adjacent to hippocampal sclerosis in a patient with TLE: a) shows abnormal clustering of neurones with NeuN marker in layer II. b) shows a band of gliosis throughout a gyrus in midlayers with GFAP marker which is shown at higher magnification in c).

Tumours and epilepsy

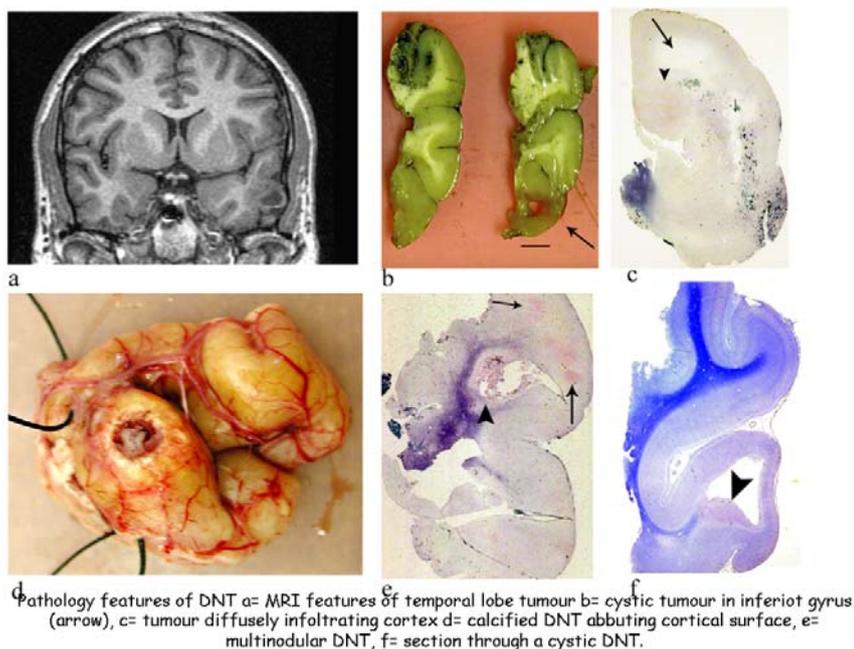
A wide variety of tumour types, particularly where there is cortical extension, can manifest clinically with focal seizures (for review see Thom et al⁹⁷). Within this group, patients with longer histories of early onset, focal or partial epilepsy, neuroimaging and pathological studies more often identify cortically-based, slow-growing tumours. These are also termed long-term epilepsy-associated tumours (LEAT). 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 glio-neuronal tumours are common diagnosis in large surgical series.

Glioneuronal tumours

There are two main tumour types in this category, dysembryoplastic neuroepithelial tumours (DNT) and gangliogliomas. 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 astrocytoma⁹⁸, the angiocentric neuroepithelial tumour or glioma⁹⁹ and rarer glioneuronal tumours such as the papillary glioneuronal tumour¹⁰⁰, continue to be recognised, some strongly associated with long-standing epilepsy¹⁰¹. DNT are typically clinically associated with partial seizures. The pathological features of the classical DNT are characterised by a multinodular, intracortical architecture composed of cells with mixed cytological features^{102,103}. 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, can be 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'). 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 histological forms of DNT described are the diffuse or non-specific forms^{103,104}. The latter have a similar cytological composition but show more diffuse infiltration of the cortex and white matter and lack a glio-neuronal element. 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^{103,105} although there are rare reports of malignant transformation¹⁰⁶. DNT tend to lack the typical 1p/19q deletions seen in oligodendrogliomas and IDH1 mutations associated with more conventional low-grade gliomas and astrocytomas¹⁰⁷.

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 morphology¹⁰⁸. 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 DNT¹⁰³ and there are reports of glioneuronal tumours showing hybrid features between DNT and ganglioglioma^{109,110}.



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^{111,112}. Expression of stem cell epitope, CD34, has also been noted in up to 80% of gangliogliomas in the small cell component¹⁰⁸, which may be of diagnostic value. Predominantly gangliogliomas are grade I neoplasms associated with a good outcome. BRAF V600E mutations are reported in around 50% of cases. 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%)^{104,108,113}.

The prominent neuronal component of these tumours is one possible explanation for the potent epileptogenicity, among others¹¹⁴. DNT, as well as gangliogliomas, have often been reported in association with adjacent cortical dysplasia (type IIIb). The diagnosis of additional cortical dysplasia should naturally be distinguished from any disturbance of the cortical architecture due to diffuse tumour infiltration. Furthermore ‘hamartia’-like clusters of OLC, mixed with neurones, forming aggregates around 0.2–1.0 are also common findings adjacent to both DNT and gangliogliomas which may represent precursor lesions of these tumours, readily observed on

immunolabelling for stem cell marker CD34. The identification of peri-lesional cortical abnormalities in the vicinity of glioneuronal tumours raises the question not only of common biological origins but, importantly, where the intrinsic epileptogenicity arises.

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 and is the most common presenting symptom in cavernomas (79%). Seizures may be generalised or partial¹¹⁵. Common features to both AVM and cavernomas include extensive peri-lesional gliosis and tissue microhaemorrhages indicative of sub-clinical bleeds. The possible mechanisms inducing epilepsy include local ischaemia as result of arterio-venous shunting, the marked associated peripheral gliosis, haemosiderin deposition or secondary epileptogenesis occurring in the temporal lobe.

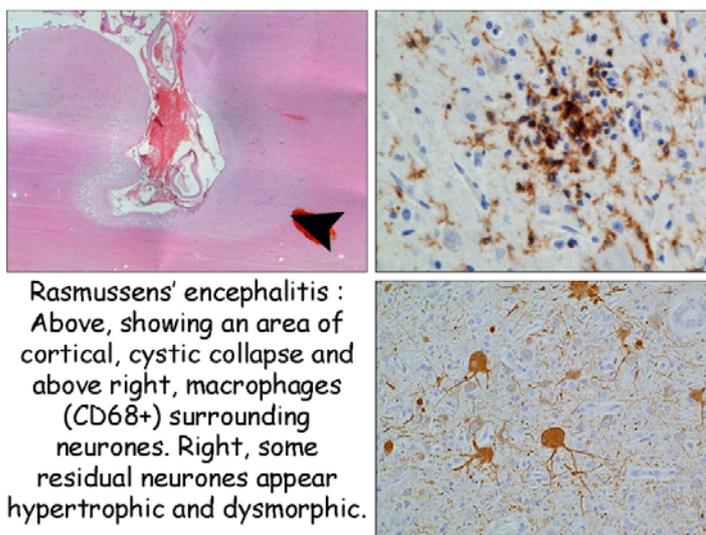
Hamartomas in epilepsy surgery

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 dysplasia¹¹⁶. The imaging characteristics of glio-neuronal hamartomas are variable^{117,118}; their lack of growth and mitotic activity help to distinguish these lesions from low-grade tumours and they tend not to recur¹¹⁶. The **hypothalamic hamartoma** has a strong association with intrinsic subcortical epileptogenesis and gelastic seizures, and may be associated with the development of secondary cortical epileptogenesis¹¹⁹. Unlike the tuberous sclerosis complex, hamartomas or malformative cortical lesions are relatively rarely reported in neurofibromatosis type 1 (NF1)¹²⁰, a syndrome in which epilepsy occurs in up to 6% of patients¹²¹. NF2 can be associated with multiple cortical glial-microhamartomas that are often incidental findings at post mortem. Interestingly, the presumed hamartomatous cell proliferation of **meningioangiomas** (MA), when associated with the NF2 complex, does not clinically manifest with seizures whereas in sporadic MA over 80% of patients present with epilepsy¹²². 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 treatment¹²³.

Autoimmune encephalitis

Patients with antibodies to intracellular or surface antigens can present with acute onset of epilepsy, psychiatric illness, cognitive decline and underlying limbic encephalitis (e.g. GAD, LGI1)¹²⁴. Rasmussen's encephalitis (RE) is a rare sporadic syndrome of presumed autoimmune 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 later stages (3 and 4) with less active inflammation and more extensive scarring¹²⁵. Inflammatory infiltrates in the cortex consist mainly of T lymphocytes (CD8>CD4+) with perivascular and perineuronal clusters¹²⁶. 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), 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 dysplasia (FCD IIIId)¹²⁵ and apoptotic neurones have been identified¹²⁶. 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^{127,128}.



Concepts of epileptogenesis in lesional neuropathologies

The term *epileptogenesis* encompasses the cascade of cellular events, following which a brain develops spontaneous seizures or epilepsy. Epileptogenesis is often divided into three stages: the acute event (the triggering insult or initial seizure), a latent period (clinically silent), and spontaneous seizures. In humans, the latent period can last for months or years. These processes are most often applied to the study of 'acquired' or symptomatic epilepsies, estimated to represent up to 50% of all epilepsies, but may also operate in genetic or idiopathic epilepsies¹²⁹. The main challenges in studying the processes of epileptogenesis in advanced-stage human tissues is to distinguish underlying pre-existing abnormalities from secondary maladaptive reorganisational changes. It is also likely that multiple epileptogenic mechanisms operate. Understanding epileptogenesis is essential to identifying new therapeutic targets. At present, most available drugs are 'anti-epilepsy' rather than 'anti-epileptogenesis'¹³⁰, but there are promising new options, modifying cellular responses that could prevent epilepsy in the first instance^{131,132}. Important areas include targeting inflammatory responses in epilepsy, blood brain barrier

dysfunction, astrocyte and neuronal generation and degeneration that could promote establishment of seizure networks.

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