Ammon's Horn Sclerosis: A Maldevelopmental Disorder Associated with Temporal Lobe Epilepsy

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Ammon's horn sclerosis (AHS) is the major neuropathological substrate in patients with temporal lobe epilepsy (TLE). Histopathological hallmarks include segmental loss of pyramidal neurons, granule cell dispersion and reactive gliosis. Pathogenetic mechanisms underlying this distinct hippocampal pathology have not yet been identified and it remains to be resolved whether AHS represents the cause or the consequence of chronic seizure activity and pharmacoresistant TLE. Whereas the clinical history indicates an early onset in most patients, ie, occurrence of febrile seizures at a young age, surgical treatment is usually carried out at an end stage of the disease. It has, therefore, been difficult to analyse the sequential development of hippocampal pathology in TLE patients. Recent molecular neuropathological studies focusing on developmental aspects of hippocampal organization revealed 2 intriguing findings in AHS specimens: *i)* The persistence of Cajal-Retzius cells in AHS patients points towards an early insult and an altered Reelin signaling pathway and *ii)* increased neurogenesis in and abnormal architectural organization of the dentate granule cell layer can be observed in young patients with early hippocampal seizure onset. These findings would be compatible with a model that involves a neurodevelopmental component in the formation of AHS. Its association with a lowered seizure threshold and an increased susceptibility for segmental cell loss in the hippocampus during the long course of the disease may constitute additional elements in a pathogenic cascade.

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Clinical and Neuropathological Findings in AHS

Large studies in patients with pharmacoresistant TLE undergoing surgical treatment of the epileptogenic area have identified Ammon's horn sclerosis (AHS; syn. mesial temporal sclerosis, hippocampal sclerosis) as the major pathological finding (6). AHS can be detected in approximately 65% of patients with TLE (Table 1). Although the pathogenesis of AHS remains to be resolved, the clinical history in most patients follows a characteristic schedule. In our series of 1109 patients obtained from 2 large European epilepsy centers, the University of Bonn Medical Center and the Institute of Neurology at the University College of London, 3 periods can be identified. Most patients presented with an initial precipitating injury before the age of 4 years. In the Bonn series, 53% of patients experienced early insults (data obtained from the clinical charts) and 47.5% of patients in London (as reviewed between the period 1996-1998). The majority of these patients in the Bonn series (70%) had a previous record of early complex febrile seizures; in the London series complex or complicated febrile convulsions were noted in 34%. This range corroborates data previously reported in the literature. Birth trauma, head injury, or meningitis were other early childhood lesions observed in TLE patients. The mean age at onset of spontaneous complex partial seizures is between 9 to 11 years. As a matter of fact, structural, molecular or functional analysis cannot visually be obtained in TLE patients during this silent period. However, the diagnosis of AHS can be verified in surgical specimens only after a long period of frustrane antiepileptic medication in most patients. The mean age at the time of surgery was 33 years with a medium history of epileptic seizures of almost 23 years (Table 1). As in most other series reported so far, both genders were equally affected in our cohort and a familial history of TLE was very rare indicating that genetic factors do not play a major role in AHS-associated TLE.

Neurosurgical resections either by two-thirds temporal lobe resection or selective amygdalohippocampectomy result in complete seizure relief (Engel class I) in more than 75% of TLE patients, and additional 12% benefit with a significant reduction of seizure frequencies (Engel class II, Table 1). The neurobiological consequences of TLE and surgical resection strategies for memory and learning processes will be discussed in the clinical contribution of this symposium (Elger, 2002).

Histopathologically, AHS is characterized by segmental pyramidal cell loss in CA1 (Sommer's sector), CA3 and CA4 (hilus), whereas CA2 pyramidal cells are

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	Bonn n = 572/725[§]	London n = 384
AHS (%)	357 (62.4%)	251 (65.4%)
Focal lesions (%)	141/143[§] (24.6 %)	95 (24.7%)
Dual pathology (%)	38 (6.6 %)	26 (6.8%)
No pathology (%)	36/10§ (6.3 %)	12 (3.1%)
AHS only		
Sex	184 f / 173 m	
Age	34.0 +/- 8.6 years	32.9* +/- 9.1 years
Age at onset of TLE	11.3 +/- 6.8 years	9.6* +/- 8.1 years
Duration of TLE	22.8 +/- 9.1 years	23.3* +/- 10.8 years
Outcome (in %)	71 / 12 / 17[$]	78/12/10[#]

Table 1. *Clinico-pathological findings in TLE patients.* f = female; m = male; Age = age of patients at surgery; Age at onset = age at onset of spontaneous seizure activity; Duration = Duration of seizure disorder before surgical treatment.

[§] 153 additional patients were collected in the Bonn series with seizure onset originating only in the lateral temporal lobe. In these samples, the surgical specimens did not contain the hippocampus.

* Clinical data for patients with AHS operated during the period 1996-1998.

[$] Postsurgical outcome has been recorded in a series of 211 patients with a mean follow up period of 91,5 month. The numbers (in %) refer to Engel class I (seizure free) / class II (one or two seizures a year) / class III-IV (seizure reduction of 75% - 50%), respectively.

[#] Post surgical outcome in 104 AHS cases operated between 1994-1999 with a minimal follow-up period of 2 years.

most seizure resistant (6). An intriguing question relates to the mechanisms of selective vulnerability between these morphologically similar neuronal cell populations. This property may be associated with abnormal neuronal circuitries. Semi-quantitative grading schemes for AHS based on the variation in the extent of principle neuronal loss in hippocampal subfields have been proposed (98). The analysis of large and unbiased series shows significant regional differences of neuronal cell loss within the CA1 subfield with variations between anterior and posterior regions or medial and lateral segments. The prosubiculum, subiculum, and presubicular areas do not usually reveal major cell damage or reactive gliosis. In contrast, the entorhinal cortex and amygdala nuclei appear to be severely affected in many, although not all AHS patients (24, 64, 101).

The dentate gyrus granule cell population reveals pathological alterations in almost 50% of patients with AHS. Lesions in this anatomical compartment range from granule cell dispersion to severe cell loss accompanied by reactive cellular and fibrillary gliosis (Figure 1). In the London series, we observed severe granule cell dispersion in 40% of AHS cases, in 10% of the cases combined with a focal or extensive bilaminar arrangement of neurons. Granule cell dispersion has been associated with early seizure onset or status epilepticus at an initial stage of the disease (41, 80). On the other hand, Mathern et al (1997) failed to establish a correlation between dentate granule cell densities and onset of seizures (59). In the London series, a positive correlation was found between the presence of granule cell dispersion and the severity of hippocampal neuronal loss, which is also reported in other studies (25, 40). This finding suggests that the processes of granule cell dispersion and AHS are closely linked. Furthermore, we have not detected an association between granule cell dispersion and the age at onset of seizures. Conversely, the occasional observation of granule cell dispersion in the absence of hippocampal cell loss but with widespread cortical malformations would rather suggest a malformative origin (38).

The access to anatomically well-preserved surgical specimens of the hippocampus obtained from clinically and neuropsychologically well-characterized TLE patients opens new possibilities for a systematic neuropathological analysis. A comparative approach correlating hippocampal tissue specimens from AHS and lesion-associated TLE as well as from various animal models (see following chapters of this symposium) has been most successful for the characterization of *i)* molecular pathways associated with spontaneous seizure activity and *ii)* pathological substrates of the epileptogenic area. The most recent and intriguing findings with respect to developmental abnormalities in the cytoarchitectural organization of the hippocampus and temporal lobe in patients with AHS will be the focus of this review.

Molecular Neuropathology of AHS

AHS has been first described in 1880 by Sommer (85) and can be observed in approximately 65% of patients suffering from TLE. A long and ongoing debate addresses the issue of whether AHS is the cause or the consequence of chronic, pharmacoresistant seizure activity. With the progress in neurosurgical treatment strategies, which make anatomically well preserved hippocampal tissue available for a systematic analysis of underlying pathomechanisms, some new features of AHS have been unraveled.

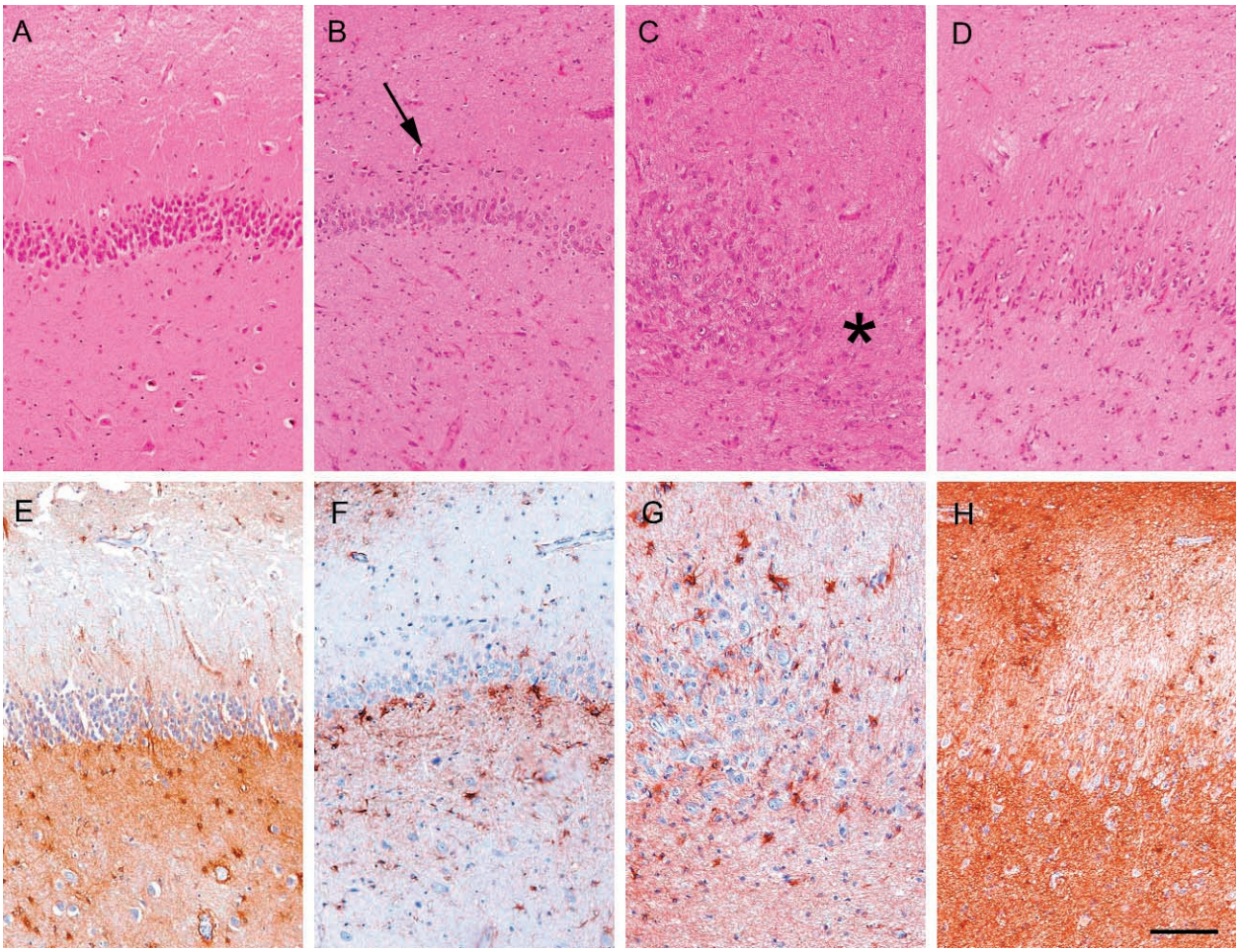

Figure 1. *The spectrum of granule cell abnormalities in AHS.* Despite segmental neuronal cell loss in the pyramidal cell layer of CA1 and CA4 (data not shown), the dentate gyrus granule cell layer shows a wide spectrum of cytoarchitectural and/or cellular damage in AHS patients. **A** – H&E staining obtained from an autopsy control patient without epilepsy. **B – D:** Obtained from different AHS patients. **B.** A smaller number of granule cells is visible accompanied by an area with focal bilamination (arrow). This example is rather discrete compared to the dramatic change observed in **C.** A bulk of dysplastic granule cells can be detected and the adjacent granule cell layer at the right side is almost shattered (asterisk). In addition, intermingled myelinated fibers are visible. **D.** In this patient, a considerable loss of the granule cell density can be scored as well as prominent astrogliosis (see also in **H**). However, there is no distinct sign of dispersion. **E–H:** Corresponding sections to A-D stained with GFAP antibodies. This panel also demonstrates considerable variability between AHS patients. Granule cell loss usually goes along with dense fibrillary gliosis (G/H), whereas moderate GFAP immunoreactivity can be observed also in the subgranular/CA4 sector in controls (**E**). Scale bar = 200 μm.

Reelin signaling pathway. The reelin molecule is an important extracellular matrix protein involved in the cytoarchitectonic guidance of migrating glia and/or neurons towards their anatomical destination (4). A mutant animal strain deficient for reelin (Reeler mouse) shows severe disturbances in cortical/hippocampal organization and layer formation (21, 23). In addition, most animals develop epileptic seizures.

Reelin molecules bind to several membrane receptors, ie, VLDL-R/ApoE-R or neuronal cadherin-like receptors (82), which induce an intracellular signaling cascade by activation of mdab1- or fyn-kinases and subsequently of CDK5 and doublecortin. Along this pathway, genetic defects in any of the genes result in a phenotype similar to the reeler mouse demonstrating their biological impact during cortical development (31).

The reelin protein is synthesized and released by a specific subpopulation of interneurons, ie, Cajal-Retzius cells. Cajal-Retzius cells are among the first neuronal populations during ontogeny with a significant decline at later stages of maturation (31). In the human hippocampus, Cajal-Retzius cells have their peak expression during the

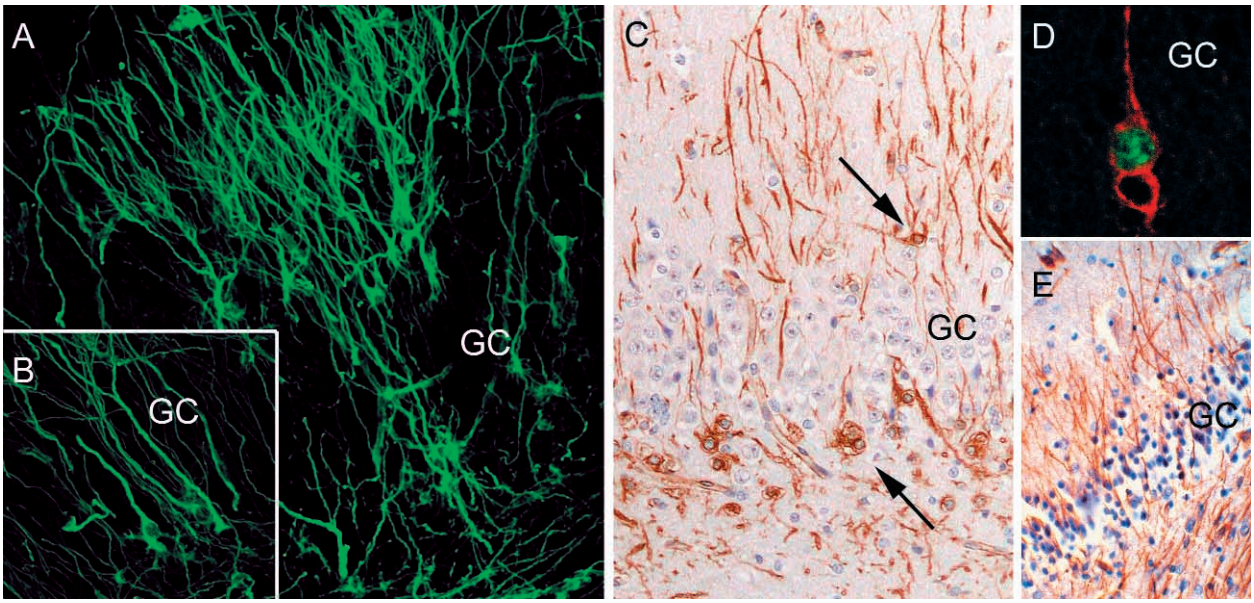

Figure 2. *Increased nestin-immunoreactive granule precursor cells in the hippocampus of TLE patients with early seizure onset.* In a group of TLE patients with early seizure onset (before age of 2 years), a significant population of nestin-immunoreactive precursor cells can be observed in the dentate gyrus. **A-B.** Confocal laser scanning microscopy. **C.** Nestin immunohistochemistry and counterstaining with haemalaun. Note the small cluster of cells in the subgranular layer (lower arrow) as well as displaced cells in the molecular layer (upper arrow). **D.** Co-localization of nestin and Ki67 identifies proliferating precursor cells. **E.** Nestin-immunoreactive precursors during hippocampal ontogeny in the human brain (obtained from the 36th week of gestation) are similar to those observed in young epilepsy patients. GC = granule cell layer.

30th to 40th week of gestation and have virtually disappeared after childhood (8). Using antibodies to reelin, as well as calretinin or calbindin (both are well recognized neuron specific calcium-binding proteins) Cajal-Retzius cells can be depicted at a typical location, ie, the most upper molecular layer of the neocortex and hippocampus/dentate gyrus. In AHS patients, a large number of these cells remain detectable even at higher age (8). A correlation between the number of persistent Cajal-Retzius cells and the clinical history showed that particular high numbers were present in patients with early complex febrile seizures. Birth trauma and meningitis were less significantly associated with a persistent population of Cajal-Retzius cells. These data strongly indicate that this important population of interneurons plays a major role in the pathogenesis of AHS. Three major conclusions may be drawn: *i)* a persistent Cajal-Retzius cell population in AHS specimens points toward an early maldevelopmental origin of AHS; *ii)* onset of early febrile seizures may be the cause or consequence of developmental abnormalities, i.e. persistent Cajal-Retzius cells; and *iii)* with our knowledge on persisting neural stem cells in the mature hippocampus, a third hypothesis would be compatible with Cajal-Retzius cells to be generated by seizure induced neurogenesis in the adult brain. However, none of the animal experiments have yet shown an increase in the number of Cajal-Retzius cells arguing against this latter possibility.

A preliminary study analyzing the distribution of reelin mRNA recognized high numbers of Cajal-Retzius cells in the mature human hippocampus (Haas et al, personal communication), whereas TLE patients with AHS and granule cell dispersion showed significantly lower levels of reelin mRNA templates. Although these findings are in contrast to previously published observations (7, 8), they underscore the potential impact of an early onset maldevelopmental basis for AHS, most likely associated with the Reelin-signaling pathway.

Neurogenesis. Proliferation and differentiation of neuronal precursors has been shown to persist in the adult mammalian CNS, in particular the subgranular matrix of the dentate gyrus (2, 15, 27, 33, 49, 50, 81). In the rat brain, hippocampal neurogenesis can be stimulated by environmental factors or learning tasks (32, 46, 78) as well as by epileptic seizures (72). However, a benefit from the structural and functional integration of newly generated neurons into a mature hippocampus by epileptic activity remains uncertain. Whereas earlier

studies proposed aberrant axonal reorganization and lowered seizure threshold as a result of an epilepsy-induced increase of granule cell neurogenesis (34, 72), subsequent studies failed to verify this hypothesis (71). Persistence of hippocampal neurogenesis can also be demonstrated in the adult human brain (27).

Histopathologic changes in human TLE specimens display features, which may point to postnatal neurogenesis, ie, dispersion of the dentate gyrus granule cell layer (40, 55) and an increase of developmentally regulated Cajal-Retzius-like cells (8). To overcome inherent problems in identifying newly generated hippocampal neurons in humans and their fate during the time course of the disease, developmental marker proteins may be helpful. In particular, the intermediate filament nestin has been associated with early neural differentiation (20, 52, 92). Nestin is expressed by multipotential neural stem cells/neuronal precursors (52). In a recent study, we observed a nestin-immunoreactive neural subpopulation in the dentate gyrus of patients with early onset temporal lobe seizures (Figure 2) (10). The cellular morphology and regional distribution of these cells are reminiscent of nestin-immunoreactive granule cell progenitors during prenatal hippocampal development. Whether these cells represent newly generated, seizure-induced granule cell progenitors or are the result of a delayed hippocampal maturation in these patients cannot be distinguished in human surgical material. Compared with recent results obtained in different animal models of limbic seizures, our data would be compatible with epilepsy associated neurogenesis in the hippocampus of young patients. DNA replication cannot be tagged for permanent labeling in human surgical material, which is a major obstacle for the identification of newly generated neurons. Proliferation activity in the nestin-immunoreactive cell population has been identified immunohistochemically using the Ki-67 epitope (Figure 2). A further study has also shown Ki67 positive cells in the subgranular layer, the site of progenitor cells in AHS specimens (22). Co-localization of Ki-67 with a subpopulation of nestin-immunoreactive cells supports an ongoing production of neural precursors or granule cell progenitors within the dentate gyrus of young TLE patients.

Clusters of nestin-immunoreactive cells within the molecular layer of the dentate gyrus represent another striking observation in young TLE patients. Their distribution would be compatible with granule cell dispersion or bi-lamination (Figure 1). Most authors have proposed perinatal or early postnatal events as pathogenic insults leading to granule cell dispersion (40, 56, 58). In particular, early seizures before the age of 4 years highly correlate with granule cell layer disorganization. The observation of supragranular nestin-immunoreactive precursor cells may indicate a role of progenitors in the development of this cytoarchitectural abnormality. An anatomical connectivity of newly generated granule cells remains to be examined, although a contribution to aberrant mossy fiber sprouting appears unlikely in rats (71). However, our data are in line with the hypothesis that early seizure onset induces abnormal neurogenesis and cytoarchitectural organization patterns of the dentate gyrus, and these changes may significantly contribute to the development of chronic TLE.

An important question remains to be addressed. Nestin-immunoreactive precursor cells have not been detected within the dentate gyrus of adult TLE patients ([10]; Thom et al, unpublished observation). This finding would correspond to a depletion of the pool of neural precursor cells due to prolonged stimulation by chronic seizure activity and may explain patterns of severe loss of granule cells in TLE patients with a long history of chronic limbic seizures. Furthermore, stereological quantification of granule cells in AHS cases has shown increased numbers in regions of maximal cell dispersion which supports the hypothesis that increased granule cell proliferation had occurred at an earlier stage (unpublished observations).

A Pathogenetic Model for AHS-associated TLE

Molecular-neuropathological studies of surgical hippocampus specimens obtained from patients with chronic TLE have focused on different pathogenetic mechanisms, such as *i)* epilepsy-associated neurogenesis, *ii)* structural (axonal/dendritic) and molecular reorganization patterns (neurotransmitter receptors, extracellular matrix), *iii)* activity dependent changes in neuronal function/synaptic plasticity (voltage dependent ion channels), *iv)* gliosis (spatial ion buffering capacity/gap junctions), and *v)* neuronal degeneration (CA1 pyramidal cell loss). However, a comprehensive model encompassing the chronic disease history in individual patients has been difficult to obtain. Considering the major clinical milestones and molecular-pathological and pathophysiological changes observed at the end stage of the disease (when neurosurgically resected hippocampal specimens are available) the following pathogenic model of AHS associated TLE can be proposed (Figure 3).

We conclude that AHS is a maldevelopmental disorder affecting the organization of the mesial temporal lobe with the dentate gyrus as the primary target. We can-

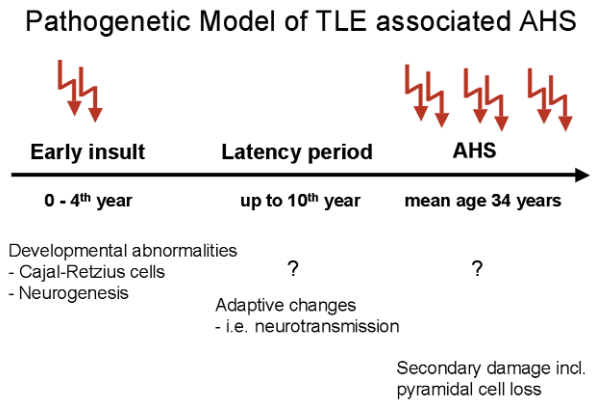

Figure 3. *Pathogenetic model of AHS associated TLE.* This pathogenic model supports the idea of a maldevelopmental origin of AHS associated TLE. The lightning symbols indicate epileptic seizure activity at different time points.

Question marks: Conclusive data on pathogenic mechanisms associated with the latency period and fullblown AHS remain to be shown.

not yet exclude that a genetic component plays a role, ie, affecting neurodevelopmental signaling pathways such as the reelin cascade. However, increased neurogenesis and/or persistence of Cajal-Retzius cells in TLE patients with AHS point towards a prolonged and abnormal maturation period. The frequent association with febrile seizures during early childhood may also relate to focal hippocampal maldevelopment. This does not rule out the possibility that early precipitating injuries may themselves initiate an abnormal hippocampal maturation profile. However, the latter model is less suited to explain the occurrence of unilateral hippocampal pathology in the majority of TLE patients.

During a latency period, which usually extends to the "teenager period," a number of structural and molecular reorganization mechanisms can be assumed. This model is difficult to address in human surgical tissue specimens obtained from an end stage of the disease. However, there is ample evidence from animal models of limbic epilepsy indicating a number of activity dependent reorganization events preceding the onset of spontaneous seizure activity. In particular, neurotransmitter receptor complexes dramatically change their molecular composition in a region-specific manner. Such modulatory changes can functionally reduce seizure threshold levels in the dentate gyrus. The loss of gate keeping functions by GABA A receptor rearrangements represents a recently established model (13, 83).

Following onset of spontaneous seizure activity within the hippocampal formation and mesial temporal lobe structures during adolescence, secondary changes associated with excitotoxic cell damage may lead to the full-blown pattern of AHS (87). This model does not rule out that segmental neuronal cell loss can occur already during an earlier period. We do, however, propose that limbic seizure activity on its own cannot induce AHS without preceding anatomical and functional alterations in the hippocampus/dentate gyrus. This assumption is supported by our studies in lesion-associated TLE, in which patients suffer from low-grade tumours, malformations or vascular lesions. In these patients, the hippocampus does not reveal substantial neuropathological changes although seizure semiologies and clinical histories can be very similar to AHS-associated TLE patients (6).

Temporal Lobe Pathology Associated with AHS

In a proportion of patients with AHS, depth electrode recordings and intraoperative electrocorticography may reflect more widespread areas of epileptiform activity involving both mesial and lateral temporal lobe regions. From neuroimaging and neuropathological studies, it is well-established that AHS can occur in combination with a second temporal lobe epileptogenic pathology such as Focal Cortical Dysplasia (FCD) or low grade glio-neuronal tumors (9, 14, 51, 53, 54, 76, 97). There are also occasional reports of distinct hippocampal malformations occurring with AHS (5) and structural hippocampal abnormalities on MRI which appear to preceed AHS (28, 35). In our 2 surgical series, dual pathologies were seen in 6.6% and 6.8% of cases. There is some evidence that less severe hippocampal neuronal loss occurs when a second pathology is present (59, 69). In general, TLE with focal mass lesions in the absence of hippocampal cell loss provides an important surgical comparison group for "classical" AHS in neuropathologial studies.

In cases with dual pathologies, "kindling" of the hippocampus by the adjacent temporal lobe lesion may account for the observed neuronal loss. Some data suggest that progressive hippocampal atrophy occurs with longer duration of seizures (29, 43, 87). It has been shown, however, that surgical removal of both lesions results in the best postoperative seizure outcome for dual pathologies (54), indicating that each component contributes to the genesis of seizures. The coincidence of dual temporal lobe pathologies also raises the important question of a common predisposing malformative process for both lesions. Furthermore, in a larger proportion of TLE cases, less well defined, subtle microscopic malformations (microdysgenesis) may be identified. Such alterations lend further evidence for

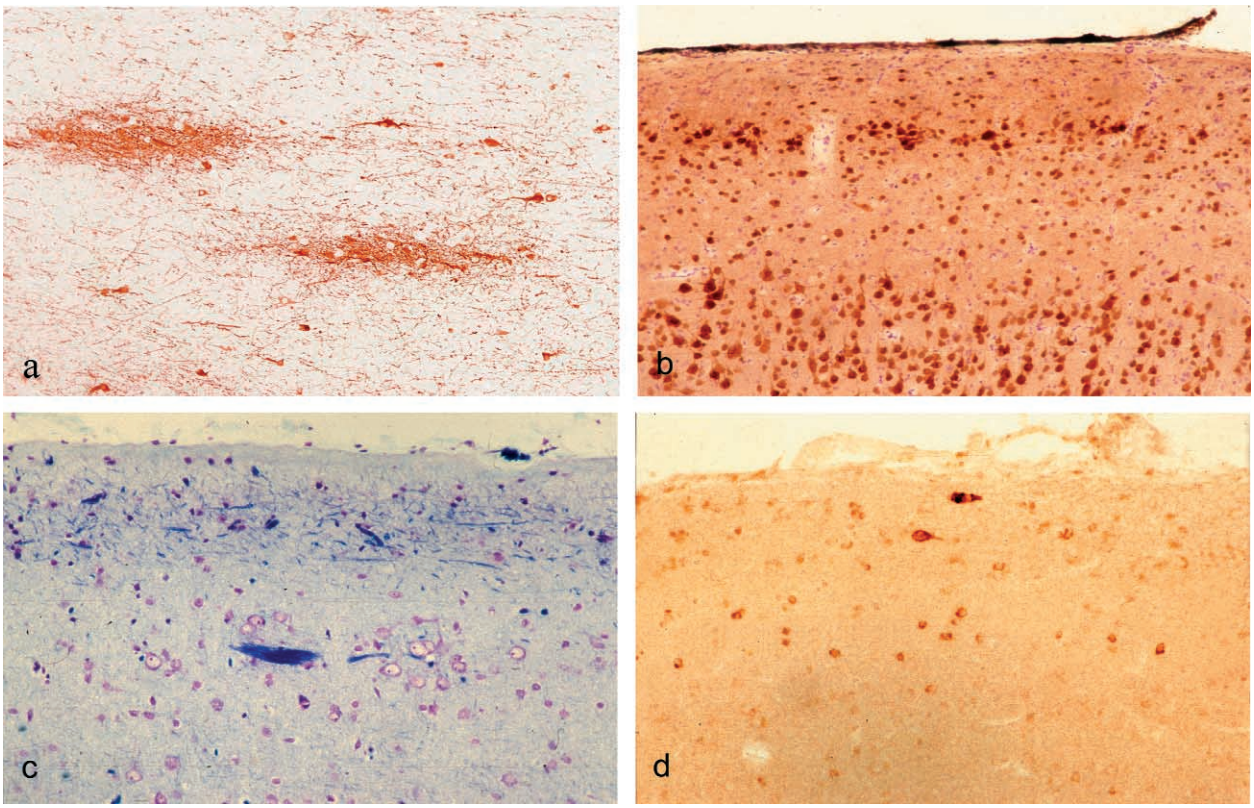

Figure 4. *Temporal lobe pathology in AHS cases.* **A.** Heterotopic white matter neurons, either in clusters or solitary (MAP2 immunostaining). **B.** Laminar neuronal loss in temporal neocortex adjacent to a hippocampus with AHS (NeuN immunohistochemistry). **C.** Abnormal myelinated fiber bundles in cortical layer I indicate a malformative origin. **D.** Persisting Cajal-Retzius cells in layer I (reelin immunostaining).

underlying temporal lobe dysgenesis which renders it more vulnerable to seizures, neuronal injury and ultimately AHS (90, 91).

Temporal lobe microdysgenesis and AHS. The term microdysgenesis (syn.: architectural dysplasia, mild cortical dysplasia, glio-neuronal hamartias [30, 44, 70, 97]) describes subtle cortical cytoarchitectural abnormalities observed in a proportion of patients with epilepsy which lack the dysplastic neuronal and glial elements that characterize FCD. In the initial descriptions of microdysgenesis in patients with idiopathic generalized epilepsy (60), the cytoarchitectural disturbances observed predominated in both the earliest and latest cortical layers to be formed. Enlarged neurons were noted in layer I (which develops from the marginal zone) and the subcortical white matter (which develops from the subplate). Ill-defined boundaries of cortical layer II were noted, the final layer formed in normal corticogenesis. The diagnosis of microdysgenesis has been viewed with scepticsm in the past as the abnormalities described are not specific to epilepsy (1, 45). Some of its features such as "a persistent columnar organization of cortical neurons," may reflect the normal anatomical cytoarchitectural variation within the temporal lobe (ie, superior temporal gyrus). However, microdysgenesis-like malformations have been shown in animal models of temporal lobe epilepsy (93). The prevalence of microdysgenesis in human TLE surgical series is largely unknown mainly due to a lack of standardized diagnostic neuropathological criteria used in different epilepsy centers (70).

White matter neuronal densities have been a focus of attention in AHS. The majority of quantitative studies confirm an excess of white matter neurons in TLE patients compared to controls with an overlap between the 2 groups (12, 26, 37, 44, 91). The density of temporal lobe white matter neurons in AHS cases covers a range from 440 to 1950/mm^3 in Nissl-stained sections employing stereological cell counting methods (12, 91). However, the criteria for abnormal, pathogenetically relevant numbers are not clearly defined (79). In normal

adult human white matter, the majority of neurons are pyramidal cells, considered to represent remnants of subplate neurons (17, 63, 75). These neurons have important roles during development, including establishing cortical architectures, gyrification and guiding thalamic connections (67, 75). Their persistence to adult life and functional roles in mature cortex are less well understood although widespread connections of these neurons with layer I have been described in rat cortex (18).

In addition to pyramidal cells, a prominent component of small white matter interneurons (<10 micron diameter) and non-pyramidal cells are also seen in TLE using NeuN or MAP2 immunostaining (Figure 4A). Subsets of these neurons label with antibodies for NPY, calbindin and calretinin, and other antibodies indicating functional heterogeneity. Excess numbers of white matter neurons in TLE may result from enhanced survival of subplate neurons, true "heterotopic" cortical neurons or even newly generated neurons. Any functional significance of these cells in relation to seizure activity remains to be determined; they may merely be the harbinger of an associated cortical malformation. Although more extensive clinico-pathological studies are required, there is some evidence that the presence of white matter microdysgenetic features in association with AHS correlates with a marginally better postoperative outcome compared to pure AHS (16, 37, 91).

Recent quantitative studies using calbindin, calretinin, and reelin immunohistochemistry have also confirmed an excess of Cajal-Retzius like cells in temporal lobe microdysgenetic malformations (30) and neocortex adjacent to AHS (91) compared to controls. In the context of the previously discussed observation of excess Cajal-Retzius cells in AHS specimens (7, 8), this may suggest a common mechanism linking AHS and temporal lobe microdysgenesis which involves the reelin pathway. Reelin protein expression persists in the mature cortex. In the adult brain, these cells may play a role in governing the formation of neuronal circuits (77). Increased numbers of Cajal-Retzius cells observed in TLE cases potentially contribute to abnormal neuronal connectivity and plasticity in epilepsy. Abnormal patterns of tangential fibers in superficial cortical laminae (Figure 4C) have also been noted in some of these patients lending further evidence for abnormal temporal lobe connectivity (90). With progress in modern MRI technologies, this issue will be more specifically addressed in the future.

Neuronal hypertrophy in AHS. Various changes of neuronal and glial morphology have been noted in studies of TLE and AHS including significant enlargement of cortical and white matter neurons (12), hypertrophy of glial cells in the temporal lobe white matter, (47, 48), residual neurons in the hippocampal hilus in AHS (11, 88) and including specific calbindin positive hilar interneurons (57). Neuronal cytomegaly and dysplasia constitute a characteristic and pathognomonic feature of FCD and are considered to represent an undifferentiated or immature phenotype (19, 65). The cellular enlargement observed in AHS has generally been considered as an adaptive response to altered metabolic demands. These neurons may support an expanded dendritic arbor and may also occur as a response to altered afferent and efferent connections in the reorganized circuitry of AHS. Although it cannot be ruled out that these hypertrophic cells correspond to an immature cell type regardless of the cause, their hypertrophic state can lead to an overestimation of neuronal densities in quantitative studies of microdysgenesis (12).

Temporal lobe atrophy and neuroimaging in AHS. Microdysgenesis has not been reliably detectable on conventional MRI scans. Therefore, a preoperative diagnosis is difficult in patients with AHS. Quantitative MRI data in AHS cohorts have suggested more widespread neocortical abnormalities in some cases (84). More recent volumetric MRI studies focusing on the temporal lobe in patients with AHS have demonstrated volume loss in proportion to hippocampal atrophy consistent with a common process involving both temporal lobe structures (68). Atrophy of neocortical gray matter has been confirmed in stereological neuropathology studies of TLE patients. These data indicate a reduction of neuropil rather than of neuronal cell numbers (12) although laminar neuronal loss in the adjacent temporal lobe can be observed in a proportion of AHS patients (90, 91).

More subtle MRI white matter abnormalities in the temporal lobe adjacent to AHS include poor demarcation between gray and white matter and increased signal intensity on T2 weighted images (16, 66). Potential neuropathological correlates for these signals are gliosis, myelin loss, perivascular atrophy, deposition of corpora amylacea as well as microdysgenesis (16, 62, 66). Proton magnetic resonance spectroscopy of the temporal lobe adjacent to AHS has shown abnormal spectra which were interpreted as correlating with myelin loss rather than gliosis in one study (61) and with microdysgenetic features in another (86). PET imaging of temporal

lobe white matter in AHS may constitute a more sensitive technique for the investigation of micro- dysgenesis. Hypometabolism (16) and increased flumazenil binding have been noticed in cases with higher white matter neuronal densities (36). However, the pathogenic substrate of temporal lobe atrophy in patients with AHS, as well as its functional impact for epileptogenesis and postsurgical outcome, need to be clarified.

Amygdala Sclerosis

Sclerosis of the amygdala (severe neuronal cell loss and concomitant gliosis) may occur in TLE patients with or without AHS (14, 39, 42, 74, 95, 101). These changes can be reproduced in animal models of status epilepticus (94). In a post mortem study of epilepsy patients with sudden death, significant neuronal loss in the lateral nucleus of the amygdala was noted both with and without a history of TLE and AHS (89).

The hippocampus, amygdala complex, and entorhinal region represent anatomically tightly connected limbic structures of the mesiotemporal lobe. Chronic seizures and mnestic deficits in patients with pharmacoresistant TLE appear to correlate with distinct patterns of histopathological alterations in these areas (101). However, the anatomical organization of these structures is difficult to assess in surgical specimens due to tissue fragmentation and poor anatomic reconstruction. Histopathological studies of the amygdala obtained from TLE patients have, therefore, concentrated on the lateral amygdala nucleus, a structure characterized by its distinct distribution of myelinated fiber bundles. Similarly, we have little information concerning histopathological alterations of the entorhinal region in temporal lobe epilepsy (24). The high rate of seizure relief following surgical removal of the hippocampus, amygdala complex as well as entorhinal region strongly suggests a pathophysiological role of these anatomic sites (73, 102). These data also indicate that 2 or more mesiotemporal regions may be involved in epileptogenesis. Because spreading pathways of epileptiform activity highly depend on anatomical projections, each focus may induce different routes of seizure propagation. Although numerous studies reported neuronal cell loss and gliosis in the amygdala of patients suffering from temporal lobe epilepsy, little attention has been paid to the lesional pattern within different anatomical subfields. In experimental models such as kindling epilepsy, amygdala nuclei are not equally prone to epileptogenesis. The most sensitive areas appear to include the amygdala-piriform cortex and central nucleus of the amygdala (which are usually not available in surgical specimens), followed by the lateral and basal nuclei of the basolateral complex. We have recently presented a combined cyto-architectonic, pigmento-architectonic, myelin-architectonic and immunohistochemical reconstruction of the amygdala, entorhinal region and hippocampus from surgical TLE specimens in order to determine their regional and cellular patterns of pathology (101). Lateral, basal, and granular subnuclei of the amygdala were consistently identified in the surgical specimens. Major histopathological alterations included neuronal cell loss as revealed by extracellular lipofuscin accumulation, glial satellitosis as well as cellular and fibrillary gliosis. The regional distribution of neuropathological changes varied considerably between different subnuclei, but the lateral nucleus was more often involved than basal and granular nuclei. These amygdala nuclei appeared to be severely affected compared to the adjacent entorhinal region. In addition, patients presenting with secondary generalized tonic-clonic seizures showed significantly more damage in mesiotemporal structures. Pathological alterations in the amygdala and entorhinal region were found to be associated with AHS in most but not all cases.

Published reports on amygdala pathology revealed striking variability (approximately 35-76% of TLE patients). In our study on 20 TLE patients, 55% of all patients displayed prominent neuronal cell loss and fibrillary gliosis in at least one subnucleus of the amygdala. Severe cell loss was clearly identified within both the basal and lateral nucleus in at least 2 patients. This pattern may correspond to the classical form of amygdala sclerosis (42). Based on the neuropathological examination of available surgical tissue specimens, the lateral nucleus appears to be a major target of epilepsy-associated histopathology. Spread of pathological alterations into basal subnuclei may be compatible with the functional organization of amygdala projection pathways from lateral towards medial regions (3). It remains to be resolved if changes in the amygdala represent an epilepsy-induced pathology, or if they contribute to the pathogenesis of TLE.

In the ventral area of the amygdala complex, clusters of small neurons admixed with clear cells reminiscent of oligodendrocytes can be frequently observed in both surgical and autopsy tissue specimens. This region corresponds to the paralaminar or granular nucleus (3). In previous studies, such foci have been interpreted as TLE-associated focal pathology, ie, glioneuronal hamartias (96). Interestingly, these neuronal clusters demonstrate immunoreactivity for the embryonic form of the neural cell adhesion molecule PSA-NCAM as

well as for the antiapoptotic protein bcl-2 (99, 100). We have to assume that these cells represent a normal cytoarchitectural component of the ventral amygdala with a remarkably immature phenotype. Further experiments may help to elucidate, whether mesial TLE contributes to anatomical abnormalities in these nuclei.

Conclusions

New findings on maldevelopmental abnormalities in AHS specimens have identified neuropathological patterns compatible with an early onset/delayed maturation of the cytoarchitecture and molecular organization of the hippocampus in TLE patients. This hypothesis is further supported by "minimal" dysgenetic changes in adjacent temporal lobe structures, ie, white matter, amygdala, or entorhinal region. These data provide a unique basis for future studies on developmentally regulated signaling cascades as key elements in AHS associated TLE.

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