Hippocampal sclerosis (HS) is the most common pathological substrate for temporal lobe epilepsy with a characteristic pattern of loss of principle neurons primarily in CA1 and hilar subfields. Other cytoarchitectural abnormalities have been identified in human HS specimens, including dispersion of dentate granule cells and cytoskeletal abnormalities in residual hilar cells. The incidence of these features, their relationship to the severity of HS and potential indication of underlying hippocampal maldevelopment is unverified. In a series of 183 hippocampectomies we identified classical HS (grades 3 and 4) in 90% of specimens, granule cell disorganization or severe dispersion in 40% of cases with a bilaminar pattern in 10%, and cytoskeletal abnormalities in hilar cells in 55% of cases. The severity of granule cell disorganization correlated closely with the degree of hippocampal neuronal loss but not with the age at first seizure or a history of a precipitating event for epilepsy such as prolonged febrile seizures. These findings suggest that granule cell disorganization is closely linked with the progression of HS rather than a hallmark of impaired hippocampal maturation. Furthermore, stereological quantitation of granule cells showed evidence of cell loss but greater numbers in regions of maximal dispersion, which may indicate enhanced neurogenesis of these cells. Quantitation of reelin-and calretinin-positive Cajal-Retzius cells in the dentate gyrus molecular layer in 26 cases showed no correlation between the number of these cells and the severity of granule cell dispersion, but increased numbers of these cells were present in HS with respect to control groups. Although a role for Cajal-Retzius cells is therefore not implicated in the mechanism of granule cell disorganization, their excess number may be indicative of underlying hippocampal maldevelopment in HS.

**Key Words:** Cajal-Retzius cells; Disorganization; Granule cells; Hippocampal sclerosis.

**INTRODUCTION**

The typical patterns of cell loss affecting specific neuronal subpopulations of the hippocampus is well defined in patients with intractable medial temporal lobe sclerosis undergoing surgery (1–4). The initiation of the cell loss and hippocampal reorganization that lead to the development of chronic seizures is less clear. Several lines of evidence suggest that an underlying maldeveloped or malformed hippocampus may predispose to the development of both febrile seizures and hippocampal sclerosis (HS). An MRI study in familial febrile convulsions has suggested a pre-existing hippocampal abnormality (5), which is also suspected in another study (6). HS has been reported in association with isolated malformation of the hippocampus in patients with partial seizures (7). In neuropathological studies the combination of HS with temporal lobe neocortical malformation, such as microdysgenesis (so called “dual pathologies”) is recognized (8–10). Furthermore, abnormal organization of the granule cells (GC) of the dentate gyrus is observed in a proportion of HS cases in temporal lobe epilepsy (TLE) (11–15), although it remains unclear whether this represents part of a pre-existing hippocampal malformation (11, 12, 16) or is a consequence of the hippocampal damage (13–15). Finally, hilar neurons with morphology similar to dysplastic neurons have been identified in HS (17, 18) in addition to an abnormal persistence of Cajal-Retzius cells in the hippocampus (19, 20), both of which may signify abnormal development.

The aim of this neuropathological study was to review a large surgical series of HS specimens from adults with intractable epilepsy and to investigate the frequency of cytoarchitectural changes that may indicate an underlying hippocampal malformation, and to relate this to the extent of hippocampal damage and aspects of the patients’ seizure history.

**MATERIALS AND METHODS**

**Case Selection**

Two hundred and six anterior temporal lobectomy and hippocampectomy specimens were included for review in this study from patients undergoing surgery at the National Hospital for Neurology and Neurosurgery between 1993 and 2000. All of the patients were adults at the time of surgery with a mean age of 31.6 yr (range 15–58 yr) and suffered medically refractory TLE. In all cases, standard pre-operative investigative protocols, including MRI measurements and EEG studies were carried out. In 183 cases a pathological diagnosis of HS was made and in these cases a second, well-defined extra-hippocampal pathology was not identified. A second group of 23 cases were
also selected in which an extra-hippocampal presumed epilepto-\textemdash\textsuperscript{genic} lesion was identified in the temporal lobe but the hippocampus was also surgically removed. These “mass lesion” cases were included to provide a comparison group to cases with HS alone. The extra-hippocampal lesions in these cases included DNT (6), ganglioglioma (2), meningoangiometatosis (1), cavernoma (4), old infarct (5), Rasmussen’s encephalitis (2), and old traumatic lesions (4), with 1 patient having both chronic encephalitis and a cavernoma. The additional investigations carried out for the purposes of this study, on tissue surplus to diagnostic requirements, have been approved by the local ethics committee for the National Hospital for Neurology and Neurosurgery (London, UK).

**Immunohistochemistry**

The hippocampal specimen was formalin-fixed for 2 to 5 days, sliced coronally at 0.4 cm intervals, routinely processed (mean: 4.5 blocks per case) and serial sections stained with H&E and Luxol fast blue with cresyl violet. After initial review, further sections were selected for Bielschowsky silver staining and GFAP (Dako, Cambridge, UK; 1:400), neurofilament (70-kDa and 200-kDa, Eurodiagnostica, Bude, UK; 1:10), and phosphorylated neurofilament (Dako, UK; 1:400) immunostaining using routine immunoperoxidase techniques. In 75 cases, Timms staining for mossy fibers was carried out. For this staining, a fresh hippocampal slice was treated in buffered 12.2% sodium sulfide solution, lightly fixed in paraformaldehyde, processed, and paraffin-embedded. Sections were cut and reacted in dark conditions with silver developer, counterstained with hematoxylin, and mounted.

**Semi-quantitative Analysis**

The severity of neuronal loss in hippocampal CA1, CA2, and hiliar subfields was assessed semi-quantitatively and graded using a modification of previously proposed systems for HS as follows (21, 22). Grade 1: Mild neuronal loss and gliosis in the hilus (end folium sclerosis). Grade 2: Mild, visually perceivable pyramidal cell loss in CA1 (which correlates with 30% cell loss [10]). Grade 3: Severe (>90%) neuronal loss in CA1 and less severe cell loss in the hilus. Grade 4: Severe neuronal loss in both CA1 and hilus. Grade 5: Severe neuronal loss in all subfields, including GC. Pyramidal cells of CA2 in cases of grade 3 and 4 HS appeared relatively preserved, but analysis of this subfield was not included in this grading scheme as in many specimens, surgical artefacts were present in this region, making assessment difficult. For the purpose of this study, all neurons within the blades of the dentate gyrus were regarded as hiliar neurons with no distinction made between CA4 and 3 pyramidal, interneurons, and cells of the polymorphic layer. Any marked variation in the grade of HS (regarded as a difference of 2 or more grades) between sections from a single case along the anterior-posterior hippocampal axis was also noted.

The architecture of the dentate granule cell (GC) layer in all the sections was examined for the presence of GC dispersion (GCD) into the molecular layer and disorganization. This was assessed on the straight sections of the dentate gyrus blades at magnification ×40 in a Leica DM RB microscope using a graticule to estimate the thickness of the cell layer in 3 places and the mean value calculated. In 6 control postmortem cases the mean width of the GC layer was 75 μm (SD = 25 μm, range 50–125 μm). In hippocampal resections the GC was categorized as normal: no significant dispersion; mild dispersion: a few single neurons in the molecular layer, separate from the main distinct GC layer, overall mean width 150–250 μm (measured in regions of maximal dispersion); severe dispersion: striking cell dispersion with a GC layer width greater than 250 μm and loss of definition from the molecular layer (Fig. 1a, b). In addition, a bilaminar GC layer (Fig. 1c), if present either extensively or focally with discrete clusters of GC in the molecular layer, was recorded as were the presence of nests of cells with the morphology of GC in ectopic locations within the hilus or CA3 (Fig. 1d, e). Evidence of deletion of the GC layer was noted, as was any variation in the appearance of the GC layer between the sections within a specimen (in the anterior-posterior axis). The width of Timms staining in the molecular layer of the dentate gyrus was measured in 3 areas with the broadest staining of mossy fiber sprouting and the mean value recorded.

**Quantitation of Cajal Retzius Cells and Granule Cells**

Twenty-six more cases of HS were further selected that showed mild GCD (n = 16), severe GCD or a bilaminar GC layer (n = 10). Sections were cut at 10 μm and immunostained with calretinin and reelin antibodies. Calretinin antibody (Swant, Switzerland) was applied at a dilution of 1:400 and incubated overnight after microwave pretreatment. The reelin antibody, a well-characterized antibody and generous gift from Prof. Goffinet, (Neurobiology Unit, University of Namur Medical School, Belgium) was applied following microwave pre-treatment of sections at a dilution of 1:200 and incubated overnight. The numbers of immunopositive cells in both calretinin and reelin sections in the molecular layer of the dentate gyrus were counted using an eyepiece graticule and a ×20 objective lens. All cells with bipolar neuronal Cajal-Retzius cell morphology were counted using an eyepiece graticule and a ×20 objective lens. All cells with bipolar neuronal Cajal-Retzius cell morphology were counted in 10 consecutive and nonoverlapping fields and the mean cell number/mm\(^2\) calculated. Identical analysis was carried out on postmortem hippocampi from 4 age-matched neurologically normal patients as controls and in 9 of the surgically removed hippocampi from patients with TLE and extra-hippocampal mass lesions.

In addition, 22 HS cases were selected that showed variation in the severity of GCD within the specimen but without perceivable GC loss. Further sections were cut at 25 μm and stained with cresyl violet/Luxol fast blue for GC quantitation. The number of GC was estimated using a stereological 3-dimensional cell counting technique (23). All GC were counted in 100-μm-wide columns perpendicular to the GC layer using a counting box (100 μm\(^2\), 10 μm deep) counting GC in consecutive, nonoverlapping boxes through the GC and molecular layer. Columns were counted in 3 different regions showing maximal GC dispersion and in 3 regions with the least or absent dispersion. The mean number of GC per column in regions of maximal versus minimal GCD was calculated and compared. Results were also compared to identical analysis carried out on postmortem hippocampal tissue from 6 age-matched, neurologically normal patients.

Details of seizure history, including age of first seizure, seizure duration (taken as the time interval between onset of habitual seizures and surgery), and any initial precipitating event...
Fig. 1. a: Hippocampal sclerosis with normal organization of the dentate granule cell layer which forms a compact cell layer approximately 100 μm thick (Luxol fast blue/cresyl violet stain, original magnification: ×72). b: Severe dispersion of the granule cells into the molecular layer of the dentate gyrus (top of figure) and nests of granule cells are also present towards the hilus (Luxol fast blue/cresyl violet preparation, original magnification: ×72). c: Bilaminar granule cell layer in hippocampal sclerosis (Luxol fast blue/cresyl violet preparation, original magnification: ×72). d: MAP2 immunostaining (Dako, UK) of neurons in the granule cell layer with prominent staining of their apical dendrites in the molecular layer and (e) clusters of cells with the morphology of GC in an ectopic location near CA3 also showing MAP2-positive but maloriented dendrites (original magnification: ×180).

RESULTS

The classical pattern of HS (grades 3 and 4) was present in over 90% of cases (Table 1) with neuronal loss predominantly in CA1 and hilar regions and with sparing for the seizures (classified as prolonged or complicated febrile seizure, meningitis, encephalitis, or trauma) were retrieved in 72 patients. Statistical analysis was carried out using paired t test, independent t-test, and Pearson’s correlation using SPSS software for Windows, version 9.
of CA2 subfield and GC. In 2.8% of cases, severe HS was present with severe cell loss in all subfields and in 1.6% of cases an "end folium" pattern of cell loss was observed with cell loss in the hilus but imperceptible cell loss in CA1 (Table 1). In patients with classical HS, a variation in the grade of cell loss in the anterior-posterior axis was marked in 4% of cases. In 73 cases of HS where Timms staining was carried out, mossy fiber sprouting in the supragranular layer (Fig. 2a) was demonstrated in all cases with classical HS, but only in 1 of 2 cases with end folium pattern of HS. Infragranular Timms staining was more often, but not exclusively, seen in cases with severe GCD. Severe dispersion of GC into the molecular layer (GCD) was seen in 40% of the specimens. A bilayer pattern of the GC layer was seen in 10% of cases, with discrete clusters or groups of GC in the molecular layer in a further 34% (Table 1). Marked variation in the severity of GCD within a specimen in the anterior-posterior axis was noted in 23% of cases. Heterotopic clusters of cells with morphology consistent with GC were seen in either the hilus or adjacent to CA3 sector in 18% of cases; this was noted both in cases with mild or severe GCD (Fig. 1d, e).

Cytoskeletal abnormalities in residual hilar neurons were noted in 55% of cases. This included enlargement or ballooning of the cell body and processes, with intense Nissl and silver staining and cytoplasmic accumulation of neurofilaments, both phosphorylated and nonphosphorylated. In some cases only occasional hilar cells demonstrated these features whereas in others, larger numbers of positively labeled neurons and cellular processes were seen (Fig. 2b). There was a correlation between the severity of GCD and severity of hippocampal sclerosis (grades 1–4, p < 0.001) (Fig. 3), the presence of cytoskeletal abnormalities in hilar neurons (p < 0.001) and the width of Timms staining in the molecular layer (p < 0.001). In 31% of cases an abnormal myeloarchitecture within the hippocampus was also noted, including horizontal fibers in the dentate GC and molecular layers and a condensation of fibers in the end folium. In 2 cases, thick bundles of myelinated fibers were observed traversing the GC layer (Fig. 2c). In the 23 cases with temporal lobe mass lesions the severity of neuronal loss in the hippocampus and mean HS grade was significantly less than in the "pure HS" cases (p < 0.001) (Table 2). The severity of GC dispersion was also significantly less (p < 0.001) as was the width of Timms staining in the molecular layer (p < 0.001). Cytoskeletal abnormalities were seen in hilar cells in only 17% and ectopic clusters of GC in the hilus or CA3 in 8.7% of cases.

Quantitative analysis of GC number in 22 cases showed a significantly higher mean GC numbers in 100 μm columns in regions with maximal (mean 42.8, SD 15.3), compared to minimal or absent GCD (mean 24.1, SD 11.3) (p < 0.001) (Table 3). Although a higher GC number was present in areas of maximal dispersion compared to controls, this was not significant (Table 3), and in only 8 of 22 cases did granule cell numbers exceed the maximum number seen in controls. The GC number in areas of minimal dispersion was, however, significantly lower than controls. Quantitation of Cajal-Retzius-like cells in the dentate gyrus molecular layer with calretinin immunohistochemistry showed fewer positive cells in cases with severe GCD or a bilaminar GC layer than cases with mild GCD, but this was not significantly different (Table 4). There was also no significant difference in the number of reelin-positive Cajal-Retzius-like cells in the 2 groups (Fig. 2d). Similar reelin-positive cells were seen in control postmortem hippocampi, although in significantly lower numbers (p < 0.05), and reelin-positive cells were also noted in the CA1 subfield and subiculum in the majority of cases. Furthermore, there were significantly fewer reelin-positive cells in the hippocampi from patients with extra-hippocampal lesions compared to HS (p < 0.001). There was a correlation in the HS cases between the number of Cajal-Retzius-like cells in the molecular layer in each case with reelin and calretinin immunohistochemistry (p < 0.05). There was no correlation between the number of reelin-positive cells and the grade of hippocampal sclerosis, duration of seizures, or the width of Timms staining in the molecular layer.

The mean age of first seizure was 4.8 yr (range 0–32 yr) and the mean duration of seizures until surgery 24.8 yr (range 0–54 yr). Initial precipitating events included a

### Summary of the data of hippocampal sclerosis cases including grade of neuronal loss (grades, 1–5, see methods) and presence of granule cell layer abnormalities

<table>
<thead>
<tr>
<th>Category (number of cases)</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
<th>Percentage of HS cases in each category for the period 1993–2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade of HS (n = 183)</td>
<td>1.6%</td>
<td>5%</td>
<td>57.2%</td>
<td>33.4%</td>
<td>2.8%</td>
<td>Percentage of all cases with bilayer arrangement 10.3% or clusters of GC in ML</td>
</tr>
<tr>
<td>Presence of GC dispersion (n = 183)</td>
<td>GC depletion</td>
<td>Normal GC layer</td>
<td>Mild GC dispersion</td>
<td>Severe GC dispersion</td>
<td>4%</td>
<td>8.6%</td>
</tr>
</tbody>
</table>
prolonged or complex febrile seizure (16.2%), head injury (8.8%), and meningitis or encephalitis (8.8%). There was no correlation with age of first recorded seizure or duration of seizures and either the grade of HS or the severity of GCD. There was no significant difference in the mean grade of HS or presence of GCD for cases with any type of initial precipitating event compared to HS without a precipitating event.

DISCUSSION

Hippocampal sclerosis has been the subject of intensive scrutiny over the last decades and yet its exact etiology remains elusive. There is epidemiological evidence that an injury occurring early in life precipitates the process of neuronal loss (24, 25), but underlying aberrant hippocampal development has also been speculated. The combination of HS with neocortical malformation is noted in some patients with epilepsy, as is malformation of the hippocampus in isolation or as part of a more widespread process (7–10, 26). In patients with hippocampal sclerosis alone, various cytoarchitectural abnormalities have been reported in neuropathological studies suggesting an underlying malformation, although their frequency, significance, and temporal relationship to the process of HS are not well defined. We aimed to address this by reviewing these abnormalities in a large surgical series of hippocampal resections for epilepsy.

In 183 HS specimens, the classical pattern was seen in 90% of cases, with neuronal loss predominant in CA1 and hilar subfields, whereas end folium and severe hippocampal sclerosis were seen in only 1.6 and 2.8% of cases, respectively. In all of the cases with classical hippocampal sclerosis in which Timms staining was performed, mossy fiber sprouting into the molecular layer was confirmed. Mossy fiber reorganization has been proposed as one of the major epileptogenic mechanisms in HS (27, 28). Furthermore, in keeping with previous observations (15), infragranular Timms staining was more prominent in cases with severe GCD.

In an earlier large study of HS, end folium sclerosis was reported in 4% of 122 cases and classical and severe HS in 57% and 39%, respectively (1). The smaller number with severe HS in the present study may reflect differences in pre-operative assessments and patient selection. By contrast, in the second smaller group of patients in our study with temporal lobe mass lesions, significantly less hippocampal neuronal loss was demonstrated, which has also been the finding in a previous study (3).

We did not find any correlation between the severity of neuronal loss in the hippocampus and clinical parameters, including whether there was any sort of initial precipitating event, age of onset of first seizure, and the duration of seizures prior to surgery. This contrasts with previous studies that have suggested that greater hippocampal neuronal loss is present where there is a history of a precipitating event, particularly if it is a seizure (29), and with earlier age of onset of seizures (30).

Disorganization of the GC layer in HS was first recognized by Houser (11, 12) and considered to most likely represent a neuronal migration disorder. Indeed, similar abnormalities have been reported bilaterally, in association with neocortical malformations, and in the absence of epilepsy and HS, lending support to this theory (16). Similarly, in animal models of cortical malformations, abnormalities of the GC layer have also been identified. In the p35 mutant mouse, heterotopic GC are present in the molecular layer and in the hilus with mossy fiber sprouting (31), and in the reeler mouse, dispersion of GC is also identified (32). In Houser’s initial series of 34 cases of surgical HS, mild to marked granule cell dispersion was seen in 38% of specimens, with a bilaminar pattern in 18% (12). In a more recent study, GCD was identified in 45% in a series of 20 (15). In the present series of 183 HS cases, we identified severe GCD in 40% with a bilaminar pattern in a further 10.3%. In essence, marked organizational abnormalities of the GC layer is present in approximately one half of HS cases. In our study, the lack of correlation between the presence of GCD and either the age of onset of seizures or history of a precipitating event make it unlikely that this laminar disorganization is related to an insult disrupting the normally prolonged maturation of the hippocampus, which is known in humans to extend into the first few years of life.

We have, however, shown a correlation between the severity of GCD and severity of neuronal loss in HS. This supports findings from previous work by Lurton and colleagues that the extent of GCD correlates with the severity of overall hippocampal neuronal loss (15). In addition, in Houser’s original work, a correlation between GCD and neuronal loss in the hilar polymorph cell layer was noted (12). These findings would seem to confirm that the degree of GCD is related to the extent of hippocampal damage, suggesting it is a secondary phenomenon occurring in the evolution of HS. Disorganizational

Fig. 2. a: Timms staining showing supragranular and, to a lesser extent, infragranular mossy fiber sprouting (original magnification: ×120). b: Hilar neurons showing marked ballooning of cytoplasm and accumulation of phosphorylated neurofilaments (immunohistochemistry for phosphorylated neurofilaments, original magnification: ×120). c: Abnormal bundles of myelinated fibers traversing the granule cell layer (Luxol fast blue/cresyl violet preparation, original magnification: ×320). d: Reelin-positive bipolar cell with the morphology of a Cajal-Retzius cells in the dentate gyrus molecular layer (reelin immunohistochemistry, original magnification: ×320).
abnormalities of the GC layer were much less frequently observed in the mass lesion epilepsy group, which also showed less severe hippocampal neuronal, supporting the hypothesis that GCD is more closely linked to the pathological process of HS rather than a manifestation of severe temporal lobe seizures.

The potential cellular signals involved in the mechanisms of GCD remain to be identified; neurotrophin overexpression during seizures having been suggested as one possibility (13, 14). Reelin and p35 are key proteins regulating normal neuronal migration and laminar organization during mammalian cortical development, with reelin protein being secreted by Cajal-Retzius cells (33, 34). An interesting study has shown an abnormal persistence of calretinin-labeled Cajal-Retzius cells in human HS (19, 20), including an excess of cells in the dentate gyrus molecular layer. Although this observation has not been replicated in all studies (35), a possible functional role of Cajal-Retzius cells and reelin in the architectural abnormalities of the GC layer in HS would seem plausible. We did not find, however, any relationship between the number of reelin-immunopositive Cajal-Retzius cells in HS cases with and without severe dispersion. We also failed to demonstrate increased expression of p35 in cases with GCD (data not shown). It is considered likely that reelin also has an essential physiological role in the adult brain contributing to the formation of neuronal circuits (36). In our study there was no correlation between reelin-positive cell number and the width of Timms mossy fiber sprouting. There were, however, significantly more reelin-positive cells in the hippocampi of epilepsy patients compared to controls and patients with extra-hippocampal lesions, confirming an apparent increase in the number of Cajal-Retzius cells in HS. As increased numbers of reelin-positive cells have been identified in layer I of the cortex in malformations of cortical development such as polymicrogyria (37) and microdysgenesis (38), the most likely explanation for the present observations would be that excess Cajal-Retzius cells represent a hippocampal malformation. Alternatively, Cajal-Retzius cells, as a “dynamic” cell population (39), could be recruited into the area of hippocampal injury as a secondary phenomenon. However, in our study there was no relationship between the number of these cells and the duration of epilepsy or the severity of neuronal loss to support this hypothesis.

A further explanation for the common finding of GCD in HS has arisen from studies of animal models of epilepsy. Increased neuronal proliferation in the dentate subgranular zone has been shown with newly generated cells being identified in ectopic locations, such as the hilus and molecular layer of the dentate gyrus (40). Neurogenesis of GC is also known to continue into adulthood in humans (41, 42), and it is therefore possible that GCD in HS is also a result of enhanced proliferation induced by seizures. There is some evidence to support the hypothesis that proliferation of granule cells occurs in humans with epilepsy, albeit at low turnover rates (43); and expression of immature proteins such as nestin has been shown in GC in young patients with TLE, supportive of ongoing cell genesis in epilepsy (44).

Quantitation of GC number in areas of dispersion in adults is another approach to demonstrate an increased cell number. We estimated mean GC number in 100-µm perpendicular columns through the GC and molecular layer using an established stereological 3-dimensional...
### Table 2

Comparison of hippocampal pathology in cases with and without associated mass lesion

<table>
<thead>
<tr>
<th>Temporal lobectomies and hippocampectomies with extrahippocampal &quot;mass lesions&quot;</th>
<th>Hippocampal sclerosis cases</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 23</td>
<td>n = 183</td>
<td></td>
</tr>
<tr>
<td>Hippocampal sclerosis grade (mean, range)</td>
<td>1.2, 1–3</td>
<td>3.3, 1–5</td>
</tr>
<tr>
<td>Normal granule cell layer</td>
<td>56%</td>
<td>8.6%</td>
</tr>
<tr>
<td>Severe dispersion or disorganization of GC layer</td>
<td>8.6%</td>
<td>40%</td>
</tr>
<tr>
<td>Percentage of cases with cytoskeletal abnormalities in hilar cells</td>
<td>17%</td>
<td>55%</td>
</tr>
<tr>
<td>Percentage with Timms-positive sprouting into the ML</td>
<td>57%</td>
<td>98%</td>
</tr>
<tr>
<td>Mean width of Timms-positive sprouting in ML (microns)</td>
<td>43</td>
<td>214</td>
</tr>
<tr>
<td>Clusters of GC in the hilus or CA3</td>
<td>8.7%</td>
<td>18%</td>
</tr>
</tbody>
</table>

Abbreviations: GC = granule cells, ML = molecular layer.

### Table 3

Estimation of granule cell numbers in 100-μm radial columns in epilepsy cases and controls

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean GC number in 100 μm radial column (range, SD)</th>
<th>Standard deviation</th>
<th>Significance within epilepsy group* or With controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epilepsy: Region of GC dispersion (n = 22)</td>
<td>42.8 (21–85)</td>
<td>15.3</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>Epilepsy: Region with no GC dispersion (n = 22)</td>
<td>24.1 (11–53)</td>
<td>11.3</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>Controls (n = 6)</td>
<td>37.15 (30–46)</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: GC = granule cells.

### Table 4

Results of counts of reelin and calretinin positive Cajal-Retzius-like cells (CRC) in the dentate gyrus molecular layer in controls, hippocampal sclerosis cases (HS) with and without severe granule cell dispersion, and TLE mass lesion cases. Significant difference between hippocampal sclerosis and controls* (p < 0.05) and between hippocampal sclerosis and TLE-extrahippocampal lesions* (p < 0.001)

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 4)</th>
<th>HS cases (n = 26)</th>
<th>TLE Extrahippocampal lesions (n = 9)</th>
<th>HS cases with severe granule cell dispersion or bilaminar pattern (n = 8)</th>
<th>HS cases with mild granule cell dispersion (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean reelin-positive CRC/mm² (range)</td>
<td>1.4* (0.4–3.6, 1.5)</td>
<td>4.4** (0.57–10, 2.5)</td>
<td>1.31* (0.4–3.2, 0.99)</td>
<td>4.7</td>
<td>4.5</td>
</tr>
<tr>
<td>Mean calretinin-positive CRC/mm² (range)</td>
<td>3.3* (0.4–9.2, 2.7)</td>
<td>1.62* (0.66–2.13, 0.54)</td>
<td></td>
<td>2.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Mean duration of seizures/years (range)</td>
<td>NA</td>
<td>23 (16–54)</td>
<td></td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Mean HS grade (range)</td>
<td>NA</td>
<td>3.2 (3–4)</td>
<td>1.6 (0–4)</td>
<td>3.25 (3–4)</td>
<td>3.1 (3–4)</td>
</tr>
<tr>
<td>Mean width of Timms staining/microns (range)</td>
<td>NA</td>
<td>280 (80–600)</td>
<td></td>
<td>330 (100–600)</td>
<td>150 (80–200)</td>
</tr>
</tbody>
</table>

Abbreviations: HS, hippocampal sclerosis; TLE, temporal lobe epilepsy; NA, not applicable.
without dispersion to control values did, however, show a significant reduction in number, confirming GC loss. It is likely that areas of GCD are also vulnerable to the mechanisms of epilepsy-mediated neuronal loss. Therefore, the observation of excess neuronal numbers in these regions of GCD may be of greater significance than initially apparent and suggestive of neurogenesis masked by superimposed neuronal loss. Further evidence in support of GC neurogenesis in HS comes from the identification of clusters of cells with apparent morphology of GC in other ectopic locations (e.g. the hilus or CA3) in 19% of our cases, as opposed to only 8% of epilepsy cases with mass lesions. In Houser’s studies, nests of hilar GC were identified in 2 of 15 cases and their presence was considered to indicate a failure of normal granule GC migration during development rather than an anatomical anomaly (11, 12). However, in light of the more recent experimental findings, it is perhaps more likely that GC in the hilar region and CA3 in HS also represent newly generated cells in response to seizures, as it has been shown that new GC can migrate far from their presumed site of origin (45). In animal models, there is considerable interest regarding the potential integration of newly generated GC into the hippocampal network and their functional contribution to hippocampal reorganization and mossy fiber sprouting (40, 45, 46), which may also be of relevance in human hippocampal epileptogenesis.

Cytoskeletal abnormalities have recently been recognized in hilar neurons in HS, including abnormal dendritic ramifications and accumulation of neurofilaments (17, 18). Their resemblance to the dysplastic neurons of cortical dysplasia has been commented upon, again raising the possibility that this represents a hippocampal malformation. Hypertrophy and dendritic abnormalities of calbindin-positive hilar interneurons has also been shown in HS (35). In the present study we identified cytoskeletal abnormalities in residual hilar neurons in 55% of HS cases but in only 17% of mass lesion TLE cases, and there was a correlation between their presence and the extent of GCD. This would suggest that hilar cytoskeletal changes more likely reflect an adaptive cellular phenomenon as a result of altered hippocampal circuitry rather than a primary abnormality. Abnormalities in the myeloarchitecture of the hippocampus were also seen in 31% of cases. Abnormal myelinated tracts within the neocortex have been identified in association with a variety of cortical malformations, including polymicrogyria, focal cortical dysplasia, microdysgenesis, and so the called “driftwood cortex” (dystopic cortical myelogenesis) (47–49). Abnormal bundles of fibers are also reported in experimental cortical malformations and considered to be of relevance in the propagation of paroxysmal activity (50). The identification of abnormal myelinated fibers traversing the dentate gyrus in 2 cases is intriguing and may indicate an underlying malformation. In the remaining cases, we consider the abnormal myeloarchitecture more likely to reflect a condensation of residual fibers in a sclerotic hippocampus or sprouting of new fibers.

In conclusion, from this large study, cytoarchitectural abnormalities are frequently observed in HS in addition to the typical patterns of neuronal loss. In surgical specimens of HS we are limited to studying an already established disease process and the confirmation of an underlying predisposing malformation becomes more problematic. With our large patient group we have, however, been able to study a wide spectrum of cases, representing varying stages of severity or snapshots in time in the progression of HS. Our analysis has shown that the degree of GCD is closely linked to the severity of neuronal loss but not to clinical parameters such as the duration of seizures. Furthermore, GCD in HS may be a manifestation of enhanced neurogenesis.

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