Bilateral reorganization of the dentate gyrus in hippocampal sclerosis

A postmortem study

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ABSTRACT

Background: Hippocampal sclerosis (HS) is the most common surgical pathology associated with mesial temporal lobe epilepsy (MTLE). HS is typically characterized by mossy fiber sprouting (MFS) and reorganization of neuropeptide Y (NPY) fiber networks in the dentate gyrus. One potential cause of postoperative seizure recurrence following temporal lobe surgery may be the presence of seizure-associated bilateral hippocampal damage. We aimed to investigate patterns of hippocampal abnormalities in a postmortem series as identified by NPY and dynorphin immunohistochemistry.

Methods: Analysis of dentate gyrus fiber reorganization, using dynorphin (to demonstrate MFS) and NPY immunohistochemistry, was carried out in a postmortem epilepsy series of 25 cases (age range 21–96 years). In 9 patients, previously refractory seizures had become well controlled for up to 34 years prior to death.

Results: Bilateral MFS or abnormal NPY patterns were seen in 15 patients including those with bilateral symmetric, asymmetric, and unilateral HS by conventional histologic criteria. MFS and NPY reorganization was present in all classical HS cases, more variably in atypical HS, present in both MTLE and non-MTLE syndromes and with seizure histories of up to 92 years, despite seizure remission in some patients.

Conclusion: Synaptic reorganization in the dentate gyrus may be a bilateral, persistent process in epilepsy. It is unlikely to be sufficient to generate seizures and more likely to represent a seizure-induced phenomenon. *Neurology*® 2009;73:1033–1040

GLOSSARY

AED = antiepileptic drug; **CA1p** = CA1-predominant hippocampal sclerosis; **CHS** = classical hippocampal sclerosis; **EFG** = end folium gliosis; **EFS** = end folium sclerosis; **GCD** = granule cell dispersion; **GCL** = granule cell layer; **HS** = hippocampal sclerosis; **MFS** = mossy fiber sprouting; **MTLE** = mesial temporal lobe epilepsy; **NPY** = neuropeptide Y; **ROI** = region of interest; **SE** = status epilepticus; **TLE** = temporal lobe epilepsy.

The first identification of hippocampal sclerosis (HS) and its association with epilepsy was through postmortem studies. HS has since been reported to be bilateral in up to 56% of postmortem cases in large series.¹ Bilateral HS was also observed at postmortem in patients with EEG evidence of temporal lobe epilepsy (TLE).² Modern views based on neuroimaging also recognize that HS associated with TLE may be a bilateral asymmetric disease process.^{3–7}

Temporal lobectomy is an effective treatment for patients with TLE and unilateral HS with around two-thirds³ having long-term seizure freedom. One explanation for surgical failure is a subtle abnormality in the contralateral hippocampus which becomes a secondary focus for seizures, a hypothesis supported by some EEG, quantitative MRI,^{4,5} and experimental model data.⁶

Studies have aimed to define cellular changes that could indicate seizure origin within the region of sclerosis. In this respect, reorganization of the dentate gyrus circuitry, including mossy fiber sprouting (MFS),⁷ neuropeptide Y (NPY) network alteration,⁸ and granule cell dispersion (GCD)⁹

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have been the focus of much attention in surgically removed tissues. Reevaluation of postmortem collections of patients with long histories of epilepsy affords an opportunity to investigate hippocampal pathologies in both hemispheres not addressed in previous studies. In particular, we focus on synaptic reorganization of the dentate gyrus to determine 1) if it might be a bilateral process, 2) its relationship with varying degrees or grades of HS and epilepsy syndrome type, and 3) if it is persistent following long histories of continued seizures or seizure freedom.

METHODS **Standard protocol approvals, registrations, and patient consents.** The project has been approved by Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology. Tissue from all postmortem cases was retained with appropriate consent.

Case selection and pathologic classification. Twenty-five cases were selected from the postmortem archives in the Division of Neuropathology at the National Hospital for Neurology and Neurosurgery from a period between 1992 and 2004. All patients had histories of refractory epilepsy and all but 2 were long-term residents at the Chalfont Centre, National Society for Epilepsy, United Kingdom, with well-documented histories. The notes and records of the patients were reviewed. The details are recorded in table 1 and table e-1 on the *Neurology*[®] Web site at www.neurology.org. We selected cases with bilateral, unilateral, or no hippocampal damage as judged by conventional histologic criteria. In addition, we included a group of 3 autopsy controls with no history of epilepsy or evidence of neuropathology.

The pattern of hippocampal neuronal loss and gliosis was assessed on single coronal sections from both left and right hippocampi at a single level (of lateral geniculate nucleus, corresponding to the hippocampal mid-body), each stained with hematoxylin-eosin, Luxol fast blue, and for glial fibrillary acid protein. The patterns of neuronal loss and gliosis were classified into 5 groups based on current criteria for surgical series.^{8,10} Included were classical hippocampal sclerosis (CHS or MTS type 1) and the atypical patterns of HS including end folium sclerosis (EFS or MTS type 3) and CA1-predominant HS (CA1p or MTS type 2). There was a further group with no HS (table 2); the lack of neuronal loss in such cases has been verified in a previous stereologic quantitative study.¹¹ We also identified a further group, end folium gliosis (EFG), showing marked gliosis in the end folium but without apparent neuronal loss.

Immunohistochemistry. Additional 7- μ m and 14- μ m sections from formalin-fixed, paraffin-embedded blocks were cut, dewaxed, rehydrated through graded alcohols, and taken to water. Endogenous peroxidase was blocked with 3% hydrogen peroxide in deionized water for 10 minutes. Sections were microwaved with Antigen Retrieval Solution (Vector, Burlingame, CA). Sections were incubated with polyclonal primary antibodies against dynorphin (14- μ m-thick sections) dilution 1:100 (AbD Serotec, Kidlington, Oxford, UK) and NPY (7- μ m-thick sections) 1:4,000 (Sigma, St. Louis, MO) overnight at 4°C or 1 hour at room temperature, respectively. Labeling was detected by Dako Envision. Staining was visualized with DAB+

(Dako). Nuclei were counterstained with hematoxylin. Negative controls were treated identically except that the primary antibodies were replaced with normal rabbit immunoglobulin or diluent. In between each step, sections were washed with PBST (phosphate buffered saline plus 0.05% Tween 20). NPY or dynorphin sections from all cases were stained during a single run. For immunofluorescence studies, sections were incubated with polyclonal rabbit dynorphin 1:100 (AbD Serotec) and polyclonal sheep NPY antibody 1:500 (Abcam, Plc, Cambridge, UK) using Alexafluor[®] secondary antibodies (Invitrogen, Paisley, UK) and tyramide signal amplification system (TSA Plus) (PerkinElmer, Bucks, UK) and counterstained with DAPI. Sections were viewed with a Zeiss laser confocal microscope (LSM 510 META).

Qualitative and quantitative analysis. The patterns of NPY and dynorphin staining in the dentate gyrus, including the presence of abnormal fiber sprouting into the molecular layer, were qualitatively graded as shown in table 2 based on previous descriptions and grading schemes in surgical material^{8,12,13} (figure 1).

GCD measurements. Images were captured at $\times 10$ magnification on hematoxylin-stained sections from 2 regions along both the straight sections of the supra and infragranular blades of the dentate gyrus where maximal dispersion of the granule cells was perceived, avoiding the angles and curvatures. Using Image Pro Plus software (version 6.2, Media Cybernetics, UK), the maximal perpendicular distance of the 5 most extreme granule cells in the molecular layer was measured from a line drawn along the basal granule cell layer in each image; the mean distance over all images was recorded. Measurements were repeated once to determine consistency.

GCL NPY fiber index. Four images of the granule cell layer (GCL) were captured at $\times 40$ magnification from a single section, selecting regions where the NPY fiber immunolabeling appeared maximal. The regions of interest (ROI) were outlined using Image Pro Plus Software to include the GCL only and to exclude the subgranular zone or inner molecular layer. RGB thresholds and light intensity were set and fixed between cases and the percentage of immunostained pixels per ROI was measured and the mean overall value recorded as an index of NPY fiber sprouting. These measurements were repeated for consistency.

Hilar NPY cell densities. Using Histometrix software (Kinetic Imaging, UK), an ROI including the entire hilus of the hippocampus was outlined from a single section, using the granule cell layer as the outer boundary and drawing the shortest line to close the 2 blades of the dentate gyrus. This ROI includes all of CA4 and some CA3 neurons, as well as interneurons in the polymorphic layer of the dentate gyrus. All fields within this region were systematically analyzed with $\times 40$ objective magnification and the number of NPY immunoreactive neuronal profiles counted and calculated per mm². Statistical analysis of data was carried out using SPSS software (version 16) using analysis of variance and Mann-Whitney tests for nonparametric data.

RESULTS The patterns of hippocampal damage and its laterality were grouped as in table 1.

Bilateral MFS and NPY changes in dentate gyrus. There was bilateral reorganization or sprouting of the mossy fiber axons as visualized with dynorphin immunohistochemistry (grade 2 or more) in 4 of 7 cases in group 1 (bilateral symmetric HS) and in 5 of 9

Table 1 Clinical and pathology data of epilepsy cases and controls

HS groups	Case no./postmortem no.	Age at death, y	Epilepsy syndrome	Left			Right		
				HS	Dynorphin grade*	NPY grade*	HS	Dynorphin grade*	NPY grade*
Group 1: bilateral symmetric patterns	1/EP054	71	Symptomatic MTLE	CHS	3	4	CHS	3	4
	2/EP036	72	Symptomatic focal epilepsy	EFS	2	2	EFS	2	2
	3/EP059	75	Unclassified (not MTLE)	EFG	1	1	EFG	1	1
	4/EP021	26	Symptomatic focal epilepsy (extratemporal)	EFG	2	1	EFG	2	1
	5/EP091	21	Symptomatic TLE	CA1p	1	1	CA1p	2	1
	6/EP008	47	Unclassified	CA1p	1	2	CA1p	2	2
	7/EP069	79	Symptomatic focal epilepsy	CHS	3	3	CHS	3	3
Group 2: bilateral asymmetric hippocampal patterns	8/EP007	65	Unclassified	CA1p	2	1	CHS	3	4
	9/EP052	84	Focal epilepsy	CHS	3	3	EFS	3	2
	10/EP038	74	Symptomatic focal epilepsy (extratemporal)	EFS	2	2	CHS	3	3
	11/EP055	96	Unclassified	CHS	3	3	CA1p	1	2
	12/EP057	87	Symptomatic focal epilepsy	CHS	3	3	EFS	2	2
	13/EP037	79	Symptomatic focal epilepsy	CA1p	3	4	EFG	1	1
	14/EP031	46	Unclassified	CA1p	1	1	EFG	2	2
	15/EP041	74	Probable focal epilepsy (not definite MTLE)	EFG	1	2	EFS	1	2
	16/EP002	75	No data	CA1p	3	2	CHS	3	4
Group 3: unilateral hippocampal patterns	17/EP031	38	MTLE	CHS	3	3	No HS	1	1
	18/EP061	69	Unclassified	CHS	2	3	No HS	1	2
	19/EP012	73	Symptomatic focal epilepsy	EFG	—	2	No HS	—	1
	20/EP047	60	Symptomatic focal epilepsy (extratemporal)	No HS	1	2	EFS	2	2
	21/EP075	62	TLE (not definite MTLE)	No HS	1	1	No HS	1	1
Group 4: no hippocampal sclerosis	22/EP058	47	TLE (not definite MTLE)	No HS	1	1	No HS	1	1
	23/EP053	63	Unclassified	No HS	1	1	No HS	1	1
	24/EP062	68	Unclassified	No HS	2	2	No HS	1	1
	25/EP039	46	Symptomatic epilepsy, uncertain whether focal or generalized	No HS	1	2	No HS	2	2
	26/EP075	62	TLE (not definite MTLE)	No HS	1	1	No HS	1	1
Group 5: controls (no epilepsy)	U3	85	—	No HS	1	1	No HS	1	1
	U4	63	—	No HS	1	1	No HS	1	1
	U8	49	—	No HS	1	1	No HS	1	1

*Hippocampal sclerosis (HS), dynorphin, NPY (neuropeptide Y) grades: this refers to the grading scheme for the patterns of hippocampal sclerosis, mossy, and NPY fiber reorganization as outlined in table 2.

EP = epilepsy postmortem; MTLE = mesial temporal lobe epilepsy; CHS = classical hippocampal sclerosis; EFS = end folium sclerosis; EFG = end folium gliosis; TLE = temporal lobe epilepsy; CA1p = CA1-predominant pattern of neuronal loss.

More detailed table available as table e-1 on the *Neurology*® Web site at www.neurology.org.

cases in group 2 (bilateral asymmetric HS) (figure 2, A and B). Bilateral NPY fiber reorganization (grade 2 or more) was observed in 4 of 7 of group 1 and 6 of 9 of group 2 although not in identical cases as the dynorphin (table 1) (figure 2, C and D). Bilateral NPY fiber reorganization was also seen in 2 of 4 cases

in group 3 (unilateral HS) and 1 of 5 cases in group 4 (no HS), but not in any controls.

In groups 2 and 3 with asymmetric or unilateral HS, MFS and NPY fiber patterns were assessed on the contralateral side with the better preserved hippocampus (as detailed in table 2); abnormal fiber

Table 2 Grading scheme for assessment of hippocampal sclerosis (HS), neuropeptide Y (NPY), and dynorphin immunohistochemistry (figure 1)

Grade	HS grade	NPY pattern grade	Dynorphin pattern grade
1	No HS	Normal pattern	Normal pattern
2	EFG/EFS: end folium gliosis/sclerosis	Distinction between IML/OML horizontal fibers visible but increased radial fiber networks in GCL and IML	Focal sprouting of dynorphin-positive fibers in IML
3	CA1p: CA1predominant neuronal loss	Loss of boundary between IML/OML; increased radial fibers in GCL and IML	Extensive sprouting of dynorphin-positive fibers throughout ML
4	CHS: classical HS	As above but increased fiber plexus in hilar region in addition	

The rows for each category do not correlate with each other.

EFG = end folium gliosis; EFS = end folium sclerosis; IML = inner molecular layer; OML = outer molecular layer; GCL = granule cell layer; ML = molecular layer.

sprouting in the inner molecular layer was noted for 9 cases with NPY and in 6 cases with dynorphin. Interhemispheric difference in NPY hilar densities was greatest for unilateral CHS (4.5/mm²) and asymmetric HS groups (3.9/mm²) but neither was significantly different from values in controls (2.8/mm²), patients without HS (1.2/mm²), or those with bilateral damage (2.1/mm²) ($p = 0.76$).

MFS and dentate changes in different patterns of HS.

Analysis of all hippocampal sections (left and right) showed that abnormalities of the dentate gyrus were significantly more often identified with severe grades of HS (as in table 2); for example, mean GCD ($p < 0.01$) and NPY grade ($p < 0.0001$) (Spearman test) (table 3).

GCD. GCD was greatest in CHS cases (mean thickness of granule cell layer 214 μ m) with more moderate degrees of dispersion in the EFS group (mean value 142 μ m). Mean GCD values in CA1p cases (118 μ m), EFG cases (116 μ m), and no-HS cases (120 μ m) were closer to control group values (109 μ m). The GCD in CHS was greater than in other groups ($p = 0.036$, corrected for multiple comparisons).

NPY pattern. Within the CHS group, the NPY grade was either 3 or 4, with the characteristic diminution of horizontal plexus of fibers in the outer molecular layer and sprouting of radial fibers in the GCL. Mean grades were between 1 and 2 for the other HS groups (table 3). In 5 of 8 cases with EFG, grade 2 NPY patterns were present. In 2 of 5 of the cases in group 4 (no HS), grade 2 NPY patterns were present, unilateral in 1 case (case 24) and bilateral in another (case 25) (figure 2E). The index of NPY staining in the GCL as a quantitative measure of new fiber sprouting showed higher values in all hippocampi in epilepsy groups compared to controls ($p < 0.001$; table 3). The NPY GCL staining index was independent of hilar NPY neuronal densities or NPY grade

(mean indices for NPY grades 1 to 4 being 0.48%, 17.8%, 9.3%, and 6.69%). NPY hilar neuronal densities were lower in the CHS cases (mean density 3.9/mm²) compared to other HS groups ($p = 0.025$). Densities of hilar NPY cells in CA1p (7.9/mm²), EFS (9.3/mm²), EFG (12.0/mm²), and no HS groups (9.9/mm²) were not different from control values (9.5/mm²) (p values of 0.09, 0.2, and 0.42).

MFS. The grade of dynorphin immunostaining (table 2) was higher with more severe HS grades as well as with the extent of GCD ($p < 0.001$). All CHS cases showed sprouting of dynorphin fibers in the molecular layer compared to 87% of EFS cases, whereas only 50% of CA1p cases showed dynorphin-positive fibers in the molecular layer. Three of 8 cases in the EFG group showed MFS with dynorphin immunostaining. In group 5 (no HS), dynorphin patterns were grade 2 focally and unilaterally in 2 cases (figure 2F). Dynorphin grades were higher in cases with higher NPY grades in all epilepsy groups ($p < 0.01$, Spearman test). Confocal imaging studies of NPY with dynorphin labeling, however, did not show colocalization of dynorphin and NPY in fibers in the molecular layer.

Clinical-pathologic observations. All 25 patients had generalized seizures; all but 2 had partial seizures and in 5 patients episodes of status epilepticus (SE) were recorded (table e-1). International League Against Epilepsy syndrome classification of epilepsy, based on review of the patient records including EEG and MRI data, was possible in 16 cases. Patients with documented SE were present in all the HS categories. The main clinical diagnosis was symptomatic focal epilepsy in 44%, with TLE in 20%. Patients with the syndrome of mesial TLE (MTLE) were present in both bilateral and unilateral HS groups. MFS (grade 2 or more) was observed in 60% and NPY fiber reorganization in 40% of patients with TLE syndromes and in 81% and 90% of patients with other symptomatic focal epilepsies. The persistence of abnormal patterns of NPY and MFS was identified in patients (e.g., case 11) with up to 92-year seizure histories. There was an observation of more antiepileptic drugs (AEDs) being taken during a patient's lifetime and higher NPY grade (Spearman test $p < 0.05$). Nine patients became seizure-free for up to 34 years prior to death. There was no significant difference in the dynorphin and NPY grades between the patients who became seizure-free vs those who remained refractory. For example, in case 18, who had been seizure-free for 20 years prior to death, grade 3 NPY was observed, and in case 12, seizure-free for 5 years, grade 3 dynorphin was seen (figure 2, A, B, G, and H).

Figure 1 Grading of immunohistochemistry staining in the dentate gyrus for neuropeptide Y and dynorphin

(A) grade 1 is equivalent to a normal staining pattern (case 17). (C) Grade 2 NPY confirms an increase in sprouted fibers in the dentate gyrus, in particular the granule cell layer (GCL), inner molecular layer (IML), and outer molecular layer (OML). However, there is still a clear demarcation between the horizontal fiber plexus density in the OML compared to the IML (case 6). (E) Grade 3 NPY shows an increase in fiber plexus, mainly running radially through the granule cell layer and into the IML and OML, obscuring any boundaries between these zones (case 18). (G) Grade 4 is similar to grade 3 but with a more extensive increase in hilar NPY sprouted fibers in addition (case 16). (B) Macroscopic coronal section: a case of unilateral hippocampal sclerosis (left side) at the level of the lateral geniculate nucleus, the approximate level that both hippocampi were sampled in all cases. Dynorphin grading for mossy fiber sprouting in dentate gyrus: (D) grade 1 dynorphin is equivalent to the normal staining pattern with labeling in the hilar region on mossy fiber terminals around neurons but no labeling in the IML (case 25). (F) Grade 2 dynorphin shows focal, patchy staining in the IML in addition to subgranular zone (case 8). (H) Grade 3 shows widespread, diffuse punctuate staining in the IML (case 8). (I) NPY: dynorphin double labeling: confocal image of the dentate gyrus in hippocampal sclerosis with mossy fiber sprouting and NPY fiber reorganization confirming a lack of colocalization between NPY (green) and dynorphin (red) in the molecular layer. Dapi (blue) = granule cell nuclei. Bars in A to D = 10 μ m, in E to G = 100 μ m, and in H = 20 μ m.

DISCUSSION Reorganization of the dentate gyrus circuitry, particularly the mossy fiber and NPY projections, is regarded as one hallmark of the epileptogenic sclerotic hippocampus removed for the management of refractory MTLE.^{7,8,13-15} In this postmortem series of patients with a variety of epilepsy syndromes, including MTLE, we have confirmed similar patterns of synaptic reorganization of the dentate gyrus, as described in surgical material. We also demonstrate for the first time that bilateral MFS and NPY fiber reorganization may occur in the human brain, can be associated with varying grades and patterns of HS, may arise in non-MTLE syndromes, and can persist through long seizure histories of over 90 years, as well as after long periods of seizure remission.

Postmortem studies of MFS have been rarely reported. In one study of a patient who died having previously had temporal lobectomy for apparent unilateral HS, the contralateral side showed atypical HS, but MFS was only observed on the resected side.¹⁶ The authors argued that this suggested that MFS was indicative of the epileptogenic hippocampus. In our series, we have also been able to confirm cases with unilateral HS associated with unilateral MFS (e.g., case 17, who was awaiting temporal lobe surgery but had a sudden death in epilepsy). We have, however, also demonstrated that MFS may frequently be bilateral in HS (in 36% of our selected series) and moreover present in the less overtly sclerotic hippocampus in asymmetric HS patterns.

Atypical (non-classical) HS patterns, such as EFS and CA1-predominant neuronal loss, were more frequently observed in this study than in surgical series.^{8,13,15} MFS was seen with all HS patterns including half of CA1p cases. In addition, we have also demonstrated bilateral MFS in patients who did not have MTLE. MFS is therefore not unique to MTLE. Furthermore, MFS appears irreversible, persisting for up to 92 years, and is seen after long periods of seizure freedom prior to death. Although subclinical seizures remain a remote possibility, one interpretation our findings is that MFS is a response to or facilitator of seizures rather than a primary epileptogenic process as has been the presumption from surgical studies of unilateral HS.^{12,17} More extensive study of MFS in experimental models of limbic seizures has enabled confirmation of its bilaterality, time course, and permanence, permitting assessment of its role in the facilitation or initiation of seizures.^{7,18,19} However, in the status epilepticus model, MFS has been observed without progression to spontaneous seizures.²⁰ Contralateral MFS has been observed following unilateral intrahippocampal kainate injection.²¹ Kindling experiments have also argued against MFS playing a crucial role in abnormal

Figure 2 Bilateral mossy fiber sprouting and neuropeptide Y changes in dentate gyrus in different HS grades

Bilateral mossy fiber sprouting in case 16: (A) right-sided classical hippocampal sclerosis (CHS) with grade 3 dynorphin and (B) left-sided CA1-predominant (CA1p) hippocampal sclerosis with grade 3 dynorphin. Bilateral NPY fiber sprouting in case 1: (C) right-sided CHS with grade 4 NPY pattern and (D) left-sided CHS with grade 4 NPY pattern. Fiber sprouting in the absence of hippocampal sclerosis in case 25: (E) grade 2/3 NPY fiber sprouting with visible hilar neurons and (F) grade 2 dynorphin pattern, both on the right side. Evidence of fiber sprouting with prolonged seizure-free periods in case 12: right-sided end folium sclerosis (EFS) (G) and left-sided CHS (H), both with mossy fiber sprouting on dynorphin staining. Prolonged seizure-free periods prior to death also occurred in cases 16 and 1, as illustrated in A to D (bars = 100 μ m).

Table 3 Pathologic data according to pattern of hippocampal sclerosis (all hippocampi from 25 cases left and right sides and 3 controls)

No.	Controls	CHS	CA1p	EFS	EFG	Epilepsy, no HS
1	3	13	8	7	8	14
Mean NPY grade	1	3.4	1.62	2.00	1.37	1.35
Mean dynorphin grade	1	2.9	1.6	2.1	1.42	1.15
Mean GCD, μ m (SD) range	108.64 (8.7) 93-116	214 (84) 133-425	117.57 (27.57) 91.24-160.81	141.97 (50.8) 88-256	116.12 (22.43) 95.12-164.14	120.65 (24.06) 83.5-158.2
Mean NPY index GCL (SD) range	0.14 (0.13) 0-0.4	6.9 (12.33) 0.25-43.75	4.87 (6.4) 0.09-17.80	13.69 (18.21) 0.64-55.61	5.9 (6.09) 1.1-19.36	2.3 (2.4) 0.11-6.45
Mean NPY hilar densities/mm ² (SD) range	9.5 (2.1) 7.5-12.9	3.9 (3.2) 0.4-12.8	7.9 (4.4) 1.4-16.7	9.5 (3.6) 1.9-13.1	12.1 (4.7) 6.4-19.1	9.9 (2.8) 4.9-15.0

CHS = classical hippocampal sclerosis; CA1p = CA1-predominant pattern of neuronal loss; EFS = end folium sclerosis; EFG = end folium gliosis; HS = hippocampal sclerosis; NPY = neuropeptide Y; GCD = granule cell dispersion; GCL = granule cell layer.

excitability.²² Some experimental models therefore support our interpretation that MFS is not necessarily epileptogenic.

NPY fiber networks show characteristic changes in surgical HS.^{8,12,23} We have confirmed bilateral NPY fiber reorganization in symmetric and asymmetric HS as shown for MFS. We also noted an association between NPY grade and the number of AEDs administered during a lifetime. Although the latter has been used as an indicator of the severity of seizures and treatment resistance, in this series it is not a reliable measure. For the older patients, few AEDs were available during their illness and there may be other reasons why only small numbers of drugs were tried.

NPY has anticonvulsant properties, and focal up-regulation may be an adaptive response in an attempt to modulate seizures.²⁴ NPY-immunoreactive fiber sprouting has been reported to correlate with loss of hilar NPY-immunoreactive neurones.^{12,23} In our series, fiber sprouting could be seen in the absence of HS (e.g., cases 18, 20, 24, and 25). In humans, it is still unclear from where the sprouted NPY fibers originate.²⁵ In animal models, NPY is aberrantly expressed in granule cells and mossy fibers.^{26,27} We have demonstrated an absence of significant colocalization of NPY and dynorphin in the dentate gyrus, in keeping with previous work showing insignificant NPY mRNA in granule cells or mossy fibers.^{28,29} In addition, NPY fiber sprouting did not appear to diminish through lifetimes of active epilepsy or seizure freedom. Overall, these findings support the view that NPY fiber sprouting occurs independently of neuronal loss.⁶ Our findings also support the hypothesis that NPY fiber sprouting and MFS most likely arise as a response to seizures, rather than being a critical factor in their generation.

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