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ORIGINAL PAPER

A new clinico-pathological classification system for mesial temporal sclerosis

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Abstract We propose a histopathological classification system for hippocampal cell loss in patients suffering from mesial temporal lobe epilepsies (MTLE). One hundred and seventy-eight surgically resected specimens were microscopically examined with respect to neuronal cell loss in hippocampal subfields CA1–CA4 and dentate gyrus. Five distinct patterns were recognized within a consecutive cohort of anatomically well-preserved surgical specimens. The first group comprised hippocampi with neuronal cell densities not significantly different from age matched autopsy controls [no mesial temporal sclerosis (no MTS); $n = 34$, 19%]. A classical pattern with severe cell loss in CA1 and moderate neuronal loss in all other subfields excluding CA2 was observed in 33 cases (19%), whereas the vast majority of cases showed extensive neuronal cell loss in all hippocampal subfields ($n = 94$, 53%). Due to considerable similarities of neuronal cell loss patterns and clinical histories, we designated these two groups as MTS type 1a and 1b, respectively. We further distinguished two atypical variants characterized either by severe neuronal loss restricted to sector CA1 (MTS type 2; $n = 10$, 6%) or to the hilar region (MTS type 3, $n = 7$, 4%). Correlation with clinical data pointed to an early age of initial precipitating injury

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(IPI < 3 years) as important predictor of hippocampal pathology, i.e. MTS type 1a and 1b. In MTS type 2, IPIs were documented at a later age (mean 6 years), whereas in MTS type 3 and normal appearing hippocampus (no MTS) the first event appeared beyond the age of 13 and 16 years, respectively. In addition, postsurgical outcome was significantly worse in atypical MTS, especially MTS type 3 with only 28% of patients having seizure relief after 1-year follow-up period, compared to successful seizure control in MTS types 1a and 1b (72 and 73%). Our classification system appears suitable for stratifying the clinically heterogeneous group of MTLE patients also with respect to postsurgical outcome studies.

Introduction

Mesial temporal sclerosis (MTS) is the most recognized finding in drug resistant, chronic temporal lobe epilepsies [5]. Tailored resection strategies including selective amygdala-hippocampectomy are established treatment modalities and offer a favourable outcome with up to 80% postoperative seizure freedom within the first 2 years [1, 2, 14, 17, 45, 47]. Clinical studies assume mesial temporal lobe epilepsy, however, as a heterogeneous entity with different etiologies and clinical histories [14, 17, 39, 46]. Hence, neuropathological investigations described different patterns of neuronal cell loss within hippocampal subfields and adjacent temporal lobe structures of surgical specimens [10, 27, 32, 34, 48] or autopsy brains obtained from patients suffering from chronic epilepsies [22, 28, 38, 44].

A reliable neuropathological classification system will be most helpful to separate distinct pathological subgroups and to better predict postsurgical outcome. A first systematic attempt to semi-quantitatively estimate hippocampal cell loss was published in 1992 by Wyler [48], referring to percentages of pyramidal cell loss within identified hippocampal subfields CA1–CA4. The Wyler-Score includes five grades, identifying classic and severe AHS as the most frequent pathologies. This grading system is practical in use, but thresholds were chosen arbitrarily and do not consider clinical histories. We applied computerized cluster analysis to define distinct neuropathological patterns of MTS. Neuronal cell densities were semi-quantitatively determined in anatomically confirmed hippocampal subfields and the dentate gyrus in a consecutive series of 178 surgically well-preserved hippocampus specimens and subsequently used for cluster analysis.

A further intriguing issue is the evaluation of determining factors on hippocampal pathology patterns and postsurgical outcome. Earlier studies provide evidence to time related factors such as epilepsy duration [17], age at epilepsy onset and the presence of an early preceding event, especially complex and prolonged febrile seizures [3, 9, 24, 26, 34, 45] as likely pathogenic mechanisms influencing the degree of MTS. Seizure frequency and severity are other parameters to be systematically revisited. A reliable classification system would be of paramount importance and should rely, therefore, on a systematic histopathological examination and the patients' clinical examination, especially postoperative seizure outcome.

Materials and methods

Subjects

Hippocampal specimens were obtained in the framework of a multi-centre study including four German centres for epilepsy surgery (University Medical Schools of Berlin, Bonn, Erlangen, and Freiburg).

During a time period from 2002 to 2005, 237 Patients were reported by the four centres to the German Epilepsy Surgery Reference Center. Of these, 59 had to be excluded due to incomplete preservation of the entire hippocampal structure. Altogether, 178 patients could be included [87 men (49%), 91 women (51%); mean age 38.4 ± 13.0 years; 93 (52%) with left- and 85 (48%) with right-sided resections]. Eight additional hippocampal specimens obtained from neurologically healthy autopsies (mean age 49 ± 6.8 years) served as controls. All patients underwent presurgical evaluation including Video-EEG monitoring, high-resolution MR imaging and neuropsychological testing [8, 20]. Temporal lobe epilepsy refractory to medication with a mesiotemporal focus was diagnosed in all cases. Surgical strategy comprised different subtypes of "tailored" anterior temporal resections including the hippocampal complex, i.e. standard anterior temporal lobectomy with amygdalo-hippocampectomy, polar temporal resection plus hippocampectomy, and "selective" amygdalo-hippocampectomy [8]. Informed consent was given from all patients included in our study for further scientific investigations.

Tissue preparation

For histopathological examination, hippocampal *en bloc* resections were dissected into 5-mm-thick slices along the anterior–posterior axis. Tissue samples from the mid hippocampal body [11] were chosen for this study, fixed overnight in 4% formalin and routinely processed into liquid paraffin. All specimens were cut at 4 µm with a microtome (Microm, Heidelberg),

stretched in water at 40°C and mounted on slides coated with silane (Langenbrinck; Emmendingen, Germany). The slides were air-dried in an incubator at 37°C overnight, deparaffined in descending alcohol concentration and stained with hematoxylin and eosin (HE). Hippocampal pyramidal neurons and granule cells of the dentate gyrus were specifically detected using immunohistochemistry for the neuronal nuclear antigen NeuN (A60, Chemicon, Temecula, USA, dilution 1:1,000, pre-treated with microwave) and an automated staining apparatus using the streptavidin–biotin method (Ventana; Strasbourg, France) and 3,3′-diaminobenzidine as chromogen as well as hematoxylin counterstaining. Due to less reliable NeuN staining in post-mortem tissue, neurons of autopsy controls were counted on HE-stained sections. To evaluate the reproducibility of both staining procedures, we repetitively compared NeuN and HE-stained surgical specimens and obtained similar neuronal densities (with standard deviations been slightly increased in the HE group) and identical MTS typing.

Neuronal cell counts

All tissue specimens were microscopically examined by the German Neuropathological Reference Center for Epilepsy Surgery in Erlangen. Semi-quantitative measurements of neuronal cell numbers were performed with a microcomputer imaging system (ColorView II CCD camera, AnalySIS imaging software, Stuttgart, Germany) equipped to a BX51 microscope (Olympus, Japan).

Immunohistochemically stained neuronal cell bodies (NeuN) were tagged on the computer screen and manually counted separately within hippocampal sectors CA1, CA2, CA3 and CA4 in four randomly placed visual fields at 200× objective magnification, representing 62,500 µm² (0.0625 mm²). Granule cells of the dentate gyrus were separately counted at 400× objective magnification in 20 randomly placed visual fields, representing 0.01 mm². For all neuronal cell counts the mean number of neurons/mm^2 was calculated and values transformed into z-scores (see Statistical analysis).

Statistical analysis

For statistical evaluation SPSS 12.0G for Windows, Version 12.01 was used. Statistical analysis was calculated using z-scores. The z-score represents, in standard deviation units, the amount a score deviates from the mean of the population from which the score is drawn [z = (score − mean of the population)/standard deviation of the population]. The mean of the normal curve is set at zero and the standard deviation unit has a value of one. This statistical calculation is particularly useful when comparing different anatomical compartments of varying cellularity, i.e. pyramidal cell versus granule cell layers.

Cluster analysis was used to identify distinct subgroups of neuropathological patterns. The analysis was based on Euclidean distance coefficients for all possible pairs of standardized cell loss (z-scores) in the different hippocampal compartments. We included all hippocampal subfields (CA1–CA4) and dentate gyrus into the analysis. The classification of cases into clusters was iteratively updated. After assignment of cases according to the cluster analysis, in a further step Discriminate Analysis was used to derive the classification rule. This linear equation allows the classification of new cases. Variance analysis with one-way ANOVA was performed to analyse influence of clinical parameters on distinct clusters. A respective software program to calculate MTS clusters from individual hippocampal cell counts will be made available on the internet platform of the neuropathological reference centre for epilepsy surgery upon request (http://www.epilepsie-register.de).

Results

Cluster analysis

Five distinct pathology patterns were recognized using cluster analysis (Fig. 1): no MTS = “no hippocampal cell loss” (n = 34, 19%), MTS type 1a = ”classic hippocampal sclerosis” (n = 33, 19%), MTS type 1b = “severe hippocampal sclerosis” (n = 94, 53%), MTS type 2 = “CA1 sclerosis” (n = 10, 6%), and MTS type 3 = “end folium sclerosis” (n = 7, 4%).

Within the first cohort of surgical specimens (no MTS), variation of mean neuronal cell densities were within the first standard deviation as compared to autopsy control values and, therefore, defined as normal (Fig. 2a). Compared to controls, mean neuronal densities for all hippocampal sectors ranged from 93.6 to 103.4% (Table 1). Twelve (35.3%) out of 34 patients of the “no MTS” group presented with a focal lesion in the vicinity of the hippocampus, most frequently a low grade glioneuronal tumour such as ganglioglioma or dysembryoplastic neuroepithelial tumour in seven cases, followed by cortical dysplasia in four cases and one case with a cavernoma. In contrast, within the MTS groups focal lesions were rare and equally distributed, including four cases with low-grade glioneuronal tumours, one case with a glio-mesodermal scar after trauma and one case with a former encephalitis.

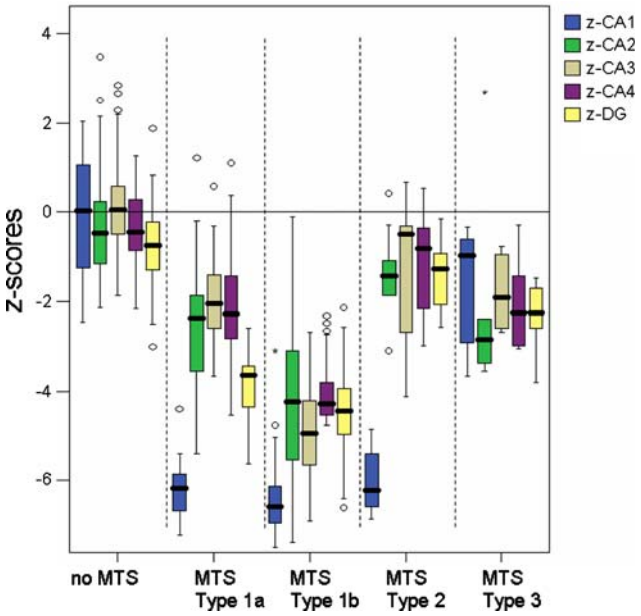

Fig. 1 Cluster analysis of histopathological findings in MTS. Semi-quantitative cell measurements were transformed into *z*-scores and revealed five distinct patterns of hippocampal pathology: no MTS = normal hippocampus with cell loss within first standard deviation compared to controls (*z*-score = 0); MTS type 1a = classic hippocampal sclerosis with severe loss in CA1 and moderate loss in remaining sectors; MTS type 1b = severe hippocampal sclerosis affecting all hippocampal sectors; MTS type 2 = severe loss in CA1 and only mild pathology within remaining sectors (i.e. CA1-sclerosis); MTS type 3 = end folium sclerosis with moderate cell loss in all sectors with the exception of CA1. *Open circles* identify individual patients, which escaped the group determining patterns. *z*-CA1-CA4 = *z*-score of CA1-CA4 field of cornu ammonis; z-DG = z-score of dentate gyrus

In MTS type 1a and 1b, the CA1 segment was most severely affected (minimum neuronal loss within the sixfold standard deviation). Cluster analysis revealed, however, significant differences between these two groups with respect to neuronal cell loss in hippocampal subfields CA2, CA3 and CA4. In MTS type 1a (Fig. 1), neuronal cell densities revealed a mean loss of 30–40% (remaining neuronal densities in CA2 = 68.7 ± 16.1%, in CA3 = 70.4 ± 15.9% and in CA4 = 61.5 ± 28.8%, Table 1), and resembled those defined earlier as classic hippocampal sclerosis [22, 38, 48] (Fig. 2d). In addition, the dentate gyrus was affected by 53.8 ± 9.7% granule cells loss. In contrast, MTS type 1b showed severe neuronal loss in all hippocampal subfields (minimum fourth standard deviation, Figs. 1, 2e), with remaining neuronal densities ranging from 38.4 ± 12.1% in DG, 14.8 ± 12.3% in CA4, 14.9 ± 9.3% in CA1 and 28.0 ± 14.5% in CA3 (Table 1) as compared to controls. Furthermore the hippocampal subfield CA2 was affected more severely with a mean neuronal density of 51.0 ± 17.7%.

MTS type 2 included ten cases with severe cell loss almost restricted to CA1 (sixfold standard deviation; Fig. 1, mean cell density 21.8 ± 9.1%, Table 1) and only mild decrease in CA2–CA4 (first to second standard deviation, Fig. 1, mean cell densities in CA2 = 82.2 ± 10.5%, in CA3 = 82.2 ± 22.5% and in CA4 = 75.7 ± 25.7%, Table 1). Due to the restricted pathology pattern observed in hippocampal subfield CA1, we considered this group as an atypical entity similar to that described earlier by de Lanerolle and coworkers [10] (Fig. 2c).

In contrast to the latter, MTS type 3 showed significant cell loss within the second standard deviation in all hippocampal subfields except for CA1 (Fig. 1). Neuronal cell loss was mainly decreased in hippocampal subfield CA4 (55.3 ± 20.5%) and the dentate gyrus (65.5 ± 11.6), whereas CA3 (70.3 ± 14.7%), CA2 (74.8 ± 26.3%) and CA1 (80.6 ± 16.8%) were only moderately affected (Table 1). According to the main pathology within the hilar region and in DG, this group was considered as end folium sclerosis [22, 48] (Fig. 2b).

Clinico-pathological correlation

Pathology clusters were correlated with a data set of previously reported clinical histories, which may have significant impact on the degree of hippocampal cell loss. Age at surgery, age at epilepsy onset, gender, side of resection and anatomical location of hippocampal specimens along the anterior–posterior axis which was used for histopathological analysis (according to Duvernoy 2005) were not different between the five MTS clusters. In addition, seizure frequency and estimated lifetime seizures did not have any influence on severity of hippocampal pathology. Intriguingly, analysis of variance revealed an influence of epilepsy duration with neuropathological patterns of MTS. Normal appearing hippocampus and MTS type 3 revealed shorter epilepsy duration compared to MTS type 1a, 1b and 2 (as characterized by severe CA1-pathology and different degrees of neuronal loss within the remaining hippocampal subfields and dentate gyrus; see Table 2).

In 171 cases, we obtained sufficient data with respect to presence/absence of initial precipitating injuries (IPIs) before epilepsy onset. Overall, 67 (39.2%) patients presented with an IPI before epilepsy onset, 38 (56.7%) of them experienced prolonged and complex febrile seizures. Other IPIs included encephalitis ($n = 15$, 22.4%), head trauma ($n = 7$, 10.4%), birth trauma ($n = 4$, 6.0%) or intracerebral bleeding ($n = 3$, 4.5%). Occurrence of an IPI was similarly distributed within MTS type 1a, type 1b and type 2 (severe

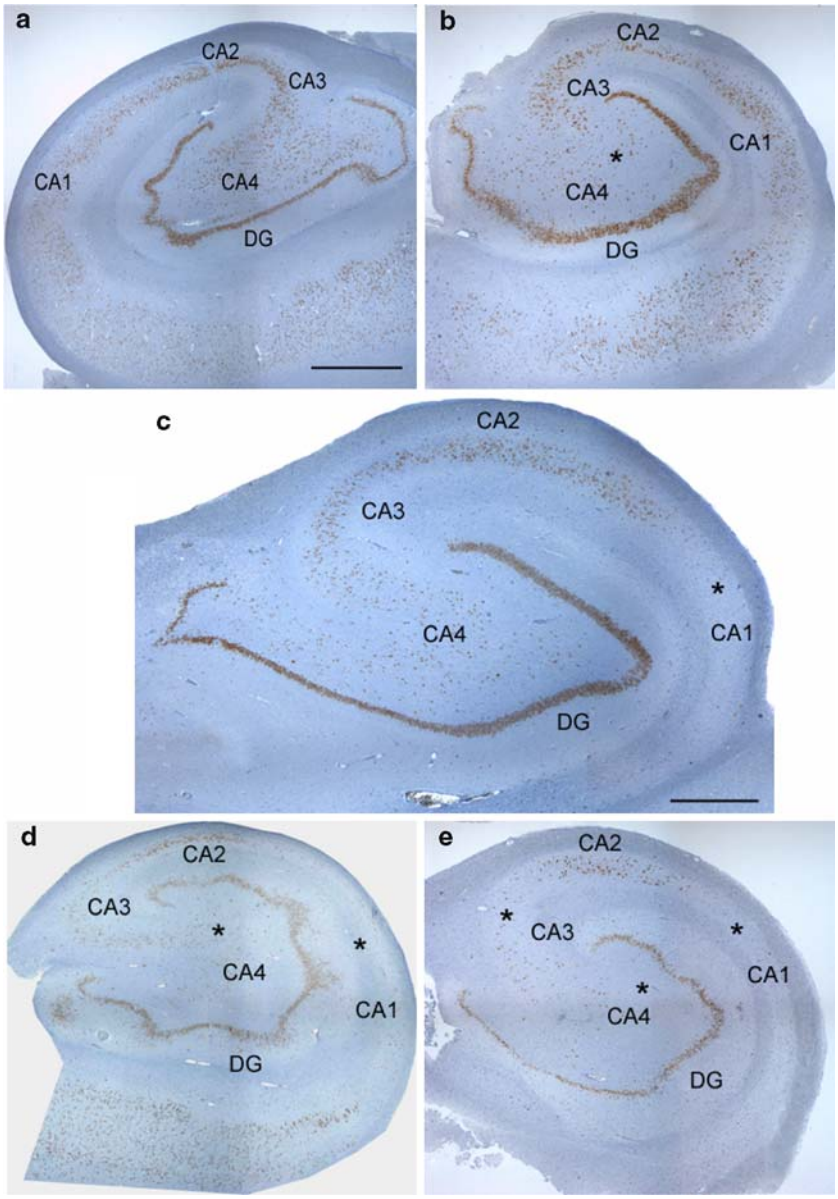

Fig. 2 Histopathological findings in MTS patients. **a** no MTS, **b** MTS type 3 (end folium sclerosis), **c** MTS type 2 (CA1-sclerosis), **d** MTS type 1a (classic hippocampal sclerosis) and **e** MTS type 1b (severe hippocampal sclerosis). *Scale bars* 1 mm. *Asterisks* index regions with predictive cell loss patterns

CA1-pathology) ranging from 43 to 50% (Table 3). In contrast, only three patients with an otherwise normal hippocampus (no MTS) experienced IPI (9.4%) and in end folium sclerosis no preceding event occurred. In the latter two groups, IPI excluded febrile seizures, while one-third of patients with MTS type 1a, type 1b and type 2 presented with febrile seizures in their early childhood ($P = 0.004$).

Precipitating injuries differed in mean age of onset ($P < 0.001$). Earliest events were birth trauma and febrile seizures, which occurred before the age of 4 years (mean 1.3 ± 0.7 years), followed by encephalitis (mean 4.9 ± 7.7 years), intracerebral bleeding (mean 12.0 ± 18.2 years) and head trauma (mean 13.3 ± 15.4 years). Detailed analysis of IPIs within different pathology groups revealed occurrence of early events such as birth trauma and febrile seizures primarily in MTS type 2 (three out of four events, 75%), MTS type 1a (11 out of 14 events, 79%) and MTS type 1b (28 out of 46 events, 61%). In patients with no MTS, only three patients presented late onset events such as head trauma, encephalitis and intracerebral bleeding. Analysis of variance confirmed the influence of age at initial precipitating injury (IPI, e.g. febrile seizure, birth trauma, encephalitis or first seizure in the absence of precipitating injury). In patients with MTS type 1a, IPI occurred at age 2 years, in MTS type 1b at age 3 years, followed by MTS type 2 at a median age of 6 years and a late event in MTS type 3 (median = 13 years) as well as normal appearing hippocampus (no MTS, median = 16 years).

Table 1 Semi-quantitative examination of surgical hippocampus specimens

Pathology		CA1	CA2	CA3	CA4	DG
No MTS	Mean	100.3	95.2	103.4	93.6	89.6
n = 34	SD	19.9	14.6	19.4	15.9	12.7
MTS type 1a	Mean	**19.0**	68.7	70.4	**61.5**	46.2
n = 33	SD	8.3	16.1	15.9	28.8	9.7
MTS type 1b	Mean	**14.9**	51	**28**	**14.8**	38.4
n = 94	SD	9.3	17.7	14.5	12.3	12.1
MTS type 2	Mean	**21.8**	82.2	82.2	75.7	80.9
n = 10	SD	9.1	10.5	22.5	25.7	11.2
MTS type 3	Mean	80.6	74.8	70.3	**55.3**	65.5
n = 7	SD	16.8	26.3	14.7	20.5	11.6

Neuronal cell densities within hippocampal subfields (CA1-CA4) and dentate gyrus (DG) expressed as percentages of autopsy control values (data not shown). Bold values highlight group identifying determinants of neuronal cell loss, i.e. CA1 in MTS type 2, and CA4 in MTS type 3

n number of cases, *SD* standard deviation, *MTS* mesial temporal sclerosis

Table 2 Correlation between histopathology and clinical histories

Pathology	*N*	Mean	SD	*P*
Age at first event or seizure				
No MTS	33	18.4	11.0	0.0001
MTS type 1a	33	5.9	8.9	
MTS type 1b	94	9.8	12.7	
MTS type 2	10	10.9	11.0	
MTS type 3	7	14.0	8.7	
Total	177	10.9	12.1	
Latency				
No MTS	33	0.0	–	0.008
MTS type 1a	33	4.4	8.5	
MTS type 1b	94	5.7	9.4	
MTS type 2	9	4.1	6.8	
MTS type 3	7	0.0	–	
Total	176	4.1	8.2	
Epilepsy duration				
No MTS	33	15.9	10.6	0.007
MTS type 1a	33	24.5	15.2	
MTS type 1b	94	25.5	14.0	
MTS type 2	9	23.2	11.4	
MTS type 3	7	17.0	9.4	
Total	176	23.1	13.8	

N = number of cases, Mean = age in years, SD = standard deviation, *P*-values refer to ANOVA (independent variable = pathology patterns, dependent variables = age at the first event, latency periods between first event and epilepsy onset, and epilepsy duration; $P < 0.05$ was defined as significant, *P*-value applies to all categories)

Six months outcome was available for 171 patients (Table 4). One hundred and thirty-five (79%) patients became seizure free (Engel 1), 18 (11%) patients showed rare disabling seizures (Engel 2), 14 (8%) patients had a worthwhile improvement (Engel 3) and four (2%) patients did not change (Engel 4). Best outcome occurred in patients with MTS type 1a (86% seizure free) and MTS type 1b (85% seizure free), followed by patients with no MTS (71% seizure free). In

Table 3 Correlation between pathology patterns and preceding events

Pathology		No event	Event	FS	Total
No MTS	*n*	29	3	–	32
	%	90.6	9.4		
MTS type 1a	*n*	17	13	10	30
	%	56.7	43.3	76.9	
MTS type 1b	*n*	47	46	25	93
	%	50.5	49.5	54.3	
MTS type 2	*n*	5	4	3	9
	%	55.6	44.4	75	
MTS type 3	*n*	7	–	–	7
	%	100			
Total	*n*	105	66	38	171
	%	61.4	38.6	57.6	100

MTS type 1a, 1b and 2 showed similar frequencies of preceding events, including febrile seizures (FS), whereas MTS type 3 and patients without MTS experienced significantly fewer early injuries ($P < 0.001$)

Table 4 Correlation between pathology patterns and postsurgical outcome

		Engel 1	Engel 2	Engel 3	Engel 4	Total
6 months after hippocampal resection						
No MTS	*n*	23	4	3	2	32
	%	71.9	12.5	9.4	6.3	
MTS type 1a	*n*	24	3	1	–	28
	%	85.7	10.7	3.6	–	
MTS type 1b	*n*	80	8	6	–	94
	%	85.1	8.5	6.4	–	
MTS type 2	*n*	5	2	2	1	10
	%	50.0	20.0	20.0	10.0	
MTS type 3	*n*	3	1	2	1	7
	%	42.9	14.3	28.6	14.3	
Total	*n*	135	18	14	4	171
	%	78.9	10.5	8.2	2.3	100
$P = 0.031$						
12 months after hippocamp resection						
No MTS	*n*	17	4	5	3	29
	%	58.6	13.8	17.2	10.3	
MTS type 1a	*n*	18	3	3	1	25
	%	72.0	12.0	12.0	4.0	
MTS type 1b	*n*	62	18	4	1	85
	%	72.9	21.2	4.7	1.2	
MTS type 2	*n*	6	1	1	1	9
	%	66.7	11.1	11.1	11.1	
MTS type 3	*n*	2	1	3	1	7
	%	28.6	14.3	42.9	14.3	
Total	*n*	105	27	16	7	155
	%	67.7	17.4	10.3	4.5	100
$P = 0.040$						

MTS types 1a and 1b gained best outcome with 85% seizure freedom after 6 months and 72% seizure relief after 12 months. However, atypical MTS type 3 was associated with the worst outcome. After 12 months only two out of seven patients remained seizure free, whereas MTS type 2 remained with a mediocre postsurgical follow-up

patients presenting with atypical variants of MTS, seizure relief was achieved only in 43% (MTS type 3) or 50% (MTS type 2) ($P = 0.031$, respectively). For 155 patients outcome data were already available after 1-year follow-up period. Patients with MTS type 1a (72% seizure free) and MTS type 1b (73% seizure free) remained the best outcome group. In contrast, MTS type 3 worsened with only 28% seizure relief 12 months after resection and MTS type 2 remained with a mediocre postsurgical outcome (Table 4).

We further examined granule cell dispersion (GCD), as microscopically determined by the recent suggestions of the ILAE commission report [46]. The normal anatomy of the dentate gyrus includes 6–8 layers of densely packed granule cells with sharp boundaries towards the pleomorphic as well as molecular layer. GCD is characterized by broadening of the dentate gyrus granule cell layer composed with more than ten layers. The boundary towards the molecular layer is blurred and ectopic granule cells can often be recognized. It is important to note that this classification should only be applied in regions without curvatures of the dentate gyrus.

Granule cell dispersion occurred in 44% of all cases (Table 5). GCD was abundant in MTS type 1a (52%) and MTS type 1b (61%). In MTS type 2 (CA1-sclerosis) only 20% of cases presented with GCD. The latter was not detectable in MTS type 3 (end folium sclerosis). It is important to note, that three patients (9%) without hippocampal neuronal cell loss showed GCD. Variance analysis with age at first event showed that presence of dispersion came along with an earlier damaging injury (mean 8.6 ± 11.0 years, median 2 years) compared to absent dispersion (mean 12.8 ± 12.7 years, median 8 years; $P = 0.022$).

Table 5 Granule cell pathology in MTS subgroups

Pathology		No dispersion	Dispersion	Total
No MTS	*N*	31	3	34
	%	91.2	8.8	
MTS type 1a	*N*	16	17	33
	%	48.5	51.5	
MTS type 1b	*N*	37	57	94
	%	39.4	60.6	
MTS type 2	*N*	8	2	10
	%	80	20	
MTS type 3	*N*	7	–	7
	%	100		
Total	*N*	99	79	178
	%	55.6	44.4	100

Granule cell dispersion predominantly occurred in MTS type 1a and 1b, whereas MTS type 3 did not reveal any granule cell layer abnormality. Note that granule cell dispersion appeared also in three patients without hippocampal cell loss (no MTS)

Discussion

Cluster analysis of microscopically examined hippocampal specimens obtained from surgically treated mesial temporal lobe epilepsies (MTLE) patients revealed five distinct neuropathological subgroups. Most intriguingly, these patterns were associated with specific clinical histories and/or postsurgical outcome, which emphasizes the suitability of our clinico-pathological classification system of MTS.

A substantial number of surgical specimens failed to demonstrate specific neuronal cell loss ($n = 34$, 19%), despite electrophysiological evidence for seizure generation within the mesial temporal lobe. This observation has been frequently reported in neuropathological surveys of similar MTS series [5, 10, 48]. The epileptogenic pathomechanisms of hippocampal seizure generation remains to be further determined in this group of patients, and we suggest a mechanism similar to the kindling animal model. Indeed, a focal lesion (low-grade glioneuronal tumours, cavernomas or cortical dysplasias) adjacent to the hippocampus was identified in 12 (35.3%) out of 34 patients without neuronal loss as potential spike generator.

A small group of patients (4%) presented with segmental cell loss mainly affecting the hilar region (CA4) and most likely correspond to end folium sclerosis described earlier [22, 48]. We identified another small cohort of patients presenting with segmental cell loss restricted to sector CA1 (CA1-sclerosis). This latter subgroup has also been described as a distinct entity by an earlier neuropathological evaluation of surgical MTS specimens (CA1 only) [10]. In our classification system, we designated these two less abundant subgroups as atypical variants of hippocampal sclerosis, i.e. MTS type 3 and MTS type 2, respectively.

The vast majority of cases, however, revealed severe cell loss of CA1 pyramidal neurons, whereas all other segments of the hippocampus proper showed neuronal cell loss to different degrees, i.e. MTS type 1. In our hands, only the degree of CA2 pyramidal cell loss determined a difference in this cohort and we differentiated them, therefore, into MTS type 1a (with moderate CA2 cell loss) and MTS type 1b (with severe CA2 cell loss). This distinction is reasonably similar to that described by Wyler et al. [48]. The Wyler-Score is well established in the neuropathological work-up of MTS and designed on the percentage of neuronal loss within hippocampal subfields CA1–CA4. Threshold values are defined either by 10% (Wyler-Score 1 = mild MTS) or 50% of neuronal loss. Classification includes also five grades (W0 = normal, W1 = mild, W2 = moderate, W3 = classical hippocampal sclerosis and W4 = severe

hippocampal sclerosis). End folium sclerosis was subsumed into W2. However, we identified end folium sclerosis and MTS predominantly restricted to CA1-sector as clinical distinct entities with a poorer postoperative seizure outcome. In previous investigations, certain difficulties evolved using the Wyler-Score to identify mild hippocampal sclerosis on the basis of 10% neuronal loss within CA1 and CA3/CA4. Our present analysis even identified 10% neuronal loss within the first standard deviation of age-matched control individuals. We would suggest, therefore, categorizing these cases into the group without histopathologically classifiable MTS (no MTS). An extension and revision of the Wyler-Score was then published by Proper [32] including mossy fibre sprouting. Mossy fibre sprouting as well as reactive gliosis are frequently associated with long-term mesial temporal lobe epilepsy [40], and were confirmed in a variety of different animal models [6, 12, 27, 29, 31, 33, 42]. Selective vulnerability of neuronal subpopulations appears also to associate with varying MTS patterns including somatostatin-, neuropeptide Y- or substance P-immunoreactive interneurons [10]. However, our histopathological classification system is deliberately restricted to histopathological parameters including pyramidal and granule cell loss, which can be microscopically determined in any pathology laboratory without further time and cost intensive or capricious neuroanatomical techniques [46], i.e. Timm staining for aberrant mossy fibre sprouting or immunohistochemical analysis of interneuronal subpopulations.

An intriguing challenge for any neuropathological classification system of CNS diseases is the relationship between cellular/molecular lesion patterns and clinical parameters as well as its predictive value for postsurgical outcome [19]. The design of our MTS classification system included, therefore, a set of clinical data, which will be retrievable in any epilepsy centre. Hallmarks of clinical histories in MTS patients are the time point of a first event (i.e. febrile seizures, head or birth trauma, encephalitis, bleeding or first seizure onset), epilepsy duration and frequency of seizures [7, 18, 36]. As reported in previous studies, seizure frequencies and age at resection, respectively duration of epilepsy, had no considerable influence on hippocampal lesion patterns [9, 23]. Our cluster analysis showed similar results (Tables 2, 3, 4). In contrast, the age of the first preceding event such as birth trauma, febrile seizures, head trauma, encephalitis or first seizure in the absence of a preceding event was significantly different in the neuropathology groups. Early events occurring under age of 7 years came along with severe neuronal loss in hippocampal subfield CA1. Moreover, earlier events under age of 3 years presented with more widespread hippocampal damage comprising all hippocampal sectors as well as the dentate gyrus. Occurrence of an event or epilepsy onset (in the absence of an event) during early adolescence did affect only the hilar region and spared also other hippocampal subfields to a greater extent. Events occurring even at later adolescence were associated with a rather normal appearing hippocampal formation. Many reports emphasize the association between initial preceding events and development of MTS [34]. The frequent association with febrile seizures in early childhood supports this hypothesis. Seizures during early childhood are associated with aberrant mossy fibre axon connections without evidence of seizure-induced cell death [25]. Moreover, prolonged seizure discharges stimulate dentate granule cell neurogenesis as a potential repair mechanism, leading to aberrant connections [31]. Such alterations may affect normal brain development and further promote epileptogenesis, whereas seizure activity during later adolescence may strengthen recurrent excitation and induce excitotoxic cell damage, i.e. MTS [29, 41]. Fig. 3.

These data confirm the earlier hypothesis that mature hippocampal networks and neurons are resistant, whereas the developing structure is more prone to epileptogenic neurotoxicity [35]. Potential pathomecha-

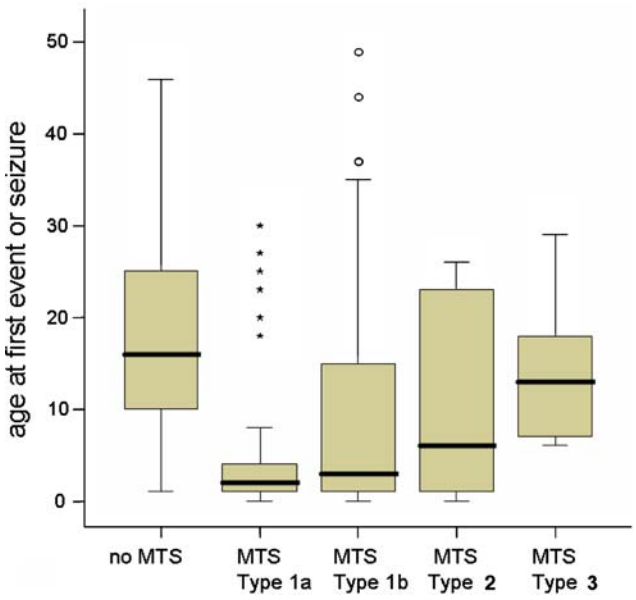

Fig. 3 Experience of initial precipitating injury (IPI) correlates with pathology patterns. *Box plots* identified an association between early onset of IPI in patients with MTS type 1a and MTS type 1b (median age 2 or 3 years, respectively). IPI occurred later in patients with atypical variants of MTS, i.e. types 2 and 3 (median age 6 years or 13 years, respectively). Patients without hippocampal cell loss experienced IPI during adolescence (median age 16 years)

nisms comprise disturbances during early brain development, which are likely to reduce the pool of neuronal adult stem cells within the hippocampal formation [4, 30]. Other possible mechanisms are increased apoptosis or reduced proliferation during brain development as well as glutamate excitotoxicity mediated by an immature neurotransmitter receptor phenotype of hippocampal cell populations including astrocytes [37]. The occurrence of GCD in nearly half of all patients with MTS type 1a and MTS type 1b also point to migratory disturbances. In this respect, we observed three patients without segmental cell loss and GCD, suggesting a malformative pathomechanism to be involved [21]. Cortical dyslamination within the temporal lobe of MTS patients can often be demonstrated supporting a more extensive developmental impairment in at least a proportion of MTS cases [15, 43].

The new MTS classification system allows some prediction of postsurgical outcome. In our large cohort of TLE patients, the overall 1-year outcome revealed a rate of 68% seizure freedom, which is in the well-recognized range of earlier reports [2, 8, 13, 16, 17]. The best outcome was achieved, however, in patients presenting with MTS type 1a and MTS type 1b (>85% seizure freedom), whereas only 28% of patients with atypical MTS pattern type 3 became seizure free. Notwithstanding, TLE patients without hippocampal neuronal loss have also a proven benefit from epilepsy surgery.

In conclusion, cluster analysis identified clinically and neuropathologically distinct patterns of hippocampal pathology. This classification system may be helpful to further study clinical determinants, i.e. psycho-physiological outcome of memory and learning performance, which is also differentially compromised in TLE patients undergoing tailored neurosurgical resections. Molecular-biological and electro-physiological efforts to distinguish MTS related pathomechanisms are likely to benefit from a systematic classification of MTS specimens and build an important basis to better understand the various MTS syndromes [46].

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