Morphological examination of ectopic neurons and synaptic reorganization in the dentate gyrus and white matter of the cerebral cortex of patients with temporal lobe epilepsy

PhD thesis

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INTRODUCTION

Epileptic seizure and epilepsy disease

Epileptic seizure is an abnormal, synchronized discharge of a neuronal population. Epileptic patients have recurrent, spontaneous epileptic seizures. 0.5-1% of the total population worldwide suffers from epilepsy (50-60 thousand people in Hungary). Electroencephalography (EEG) and brain magnetic resonance imaging (MRI) are the most important in the diagnosis of epilepsy. Epileptic seizures can be divided into two large groups: partial and generalized seizures. Partial seizures are also called focal-onset seizures, as they originate from a specific cortical area.

Temporal lobe epilepsy (TLE) is the most common type of focal epilepsy, accounting for 60-70% of adult drug-resistant epilepsies. Based on the location of the epileptogenic region, TLE is divided into mesial and lateral (neocortical) forms in the clinical practice. Mesial temporal sclerosis is caused by glial scar of the medial structures of the temporal cortex (e.g. hippocampus), which appears in hippocampal sclerosis (HS). In the background of drug-resistant TLE in adults, HS is the most common histopathological abnormality, but it can also be caused by intracranial tumor, severe head trauma, post-stroke conditions and cortical developmental abnormalities or dysgenesis. According to the latest classification of epilepsy syndromes, TLE is classified as a surgical syndrome of epilepsy. Some TLE patients do not become seizure-free by use of antiepileptic drugs (drug-resistant cases). In those cases, the brain lesion responsible for the seizures can be surgically removed (for example, HS). Of all epilepsies, surgical treatment is the most successful in TLE, when amygdalo-hippocampectomy or anterior temporal lobectomy is the method of choice.

The hippocampal sclerosis

In HS, a significant degree of cell death can be observed in certain cell groups of the hippocampus, such as the pyramidal cells of the subiculum, CA1 and CA3c regions, some interneuronal populations, hilar mossy cells and PV-immunoreactive (PV+) inhibitory cells in the GD. In addition to cell death, proliferation of some cells (glial cells and granule cells) was also observed in the hippocampus, which are probably involved in the hyperactivity of the GD. Abnormal location of granule cells (dispersion) and axon sprouting have also been described in TLE, as a result of synaptic reorganization that takes place in the affected areas, thus changing the external and internal connection system of the hippocampus. In addition to excitatory cells, sprouting of axons of axo-dendritic cells, such as calbindin containing inhibitory neurons, can

be observed. Moreover, studies described sprouting of somatostatin and NPY expressing neurons. In TLE, frequent appearance of axons of PV+ cells on the axon-initial segments of dentate granule cells was found. In the epileptic DG, strong glutamate decarboxylase immunpositivity was found in the inner one-third of the molecular layer, and it was supposed that the immunoreaction was causued by axon terminals of interneurons, including basket cells, terminating on dendrites of granule cells. The assumption, however, was not examined and verified by electron microccopy.

Neurons and synapses in subcortical white matter

It is known that a low number of neurons can be found in the subcortical white matter (WM) even in healthy people. Although the role of the so-called interstitial neurons located subcortically and in the deeper parts of the WM is not yet clear in the adults, their presence can be associated with many neurological and psychiatric disorders, including epilepsy. It is known that the synaptic release of glutamate (an excitatory neurotransmitter) is also present in WM. Synapses located in WM may play a role in the development of certain neurological and psychiatric diseases (e.g. schizophrenia, Alzheimer's disease).In TLE, large numbers of WM neurons are found in the temporal neo- and archicortex. It is not yet clear whether the increased number of WM neurons contributes to the development or maintenance of epileptic seizures, and no information is available about their synaptic connections.

AIMS OF THE STUDY

In our work, we performed morphological examinations on surgically removed samples of patients with pharmacotherapy-resistant TLE of various etiologies. During this, we examined ectopic cells and their synaptic connections in the GD and in the subcortical white matter.

In the first part of our research, we investigated the localization and axon arborization of PV+ neurons in the patients' GD. We looked for correlations between our histological results and the etiology of TLE and the clinical data of the patients.

In our laboratory, we previously showed that a large number of neurons are present in the subcortical WM, and their density is higher in TLE than in non-epileptic controls. In the second part of our research, our goal was to support the assumption that WM neurons are functionally active and have a role in the processes taking place during TLE. Therefore, we looked for a correlation between the number of WM neurons per unit area and the density of synapses in the WM. The number of neurons and the density of synapses were compared with the patients' clinical data and cognitive performance. Visualization and quantification of WM neurons was performed by detection of NeuN, a protein expressed in neurons, while visualization of synapses was possible by immunostaining synaptophysin (SYN), a glycoprotein present in the presynaptic terminals of brain and spinal cord neurons.

EXAMINATION OF PARVALBUMIN-IMMUNOREACTIVE CELLS AND AXONS IN THE HUMAN DENTATE GYRUS

MATERIALS AND METHODS

Clinical data

Surgically removed tissues (n=35) of the hippocampal formation were used in this study. TLE patients were evaluated in Department of Neurology at the University of Pécs Mecical School (UPMS) and all surgeries were performed in Department of Neurosurgery at the UPMS. In the case of 21 patients the pathological background of TLE was HS confirmed by MRI, in 7 patients the epilepsy was caused by malformation of cortical development (MCD), in 4 patients MCD coincided with HS. In three patients, the results of the MRI examination were negative, another 4 patients belonged to the group of ‘‘tumor-induced TLE group”. In addition to TLE cases, hippocampi of non-epileptic controls (n = 4) were also included in the study. Tissue samples were processed and histological evaluation has been carried out according to the institutional regulation (PTE KK RIKEB/5342). In addition, the policy of Declaration of Helsinki has been followed.

Histological processing and immunohistochemistry

After surgical removal, the tissues were immediately immersed in 4% paraformaldehyde (PFA) buffered with phosphate buffer (PB) solution, and kept for approximately 12 h at room temperature under continuous shaking. Following fixation, 10-mm-thick blocks perpendicular to the septotemporal axis of the hippocampal formation were cut, and then 80 μm sections were sectioned using a Vibratome. Immunohistochemistry was performed on free-floating sections For some sections,the primary antibody was monoclonal mouse anti-PV (1:5000, Swant, Bellizona, Switzerland), and for the other part of them, monoclonal mouse anti-NeuN (1:500, Millipore, Bellarica, MA). For detection, we used an avidin-biotin-peroxidase complex (Universal Vectastain ABC Elite Kit, Vector, Burlingame, CA), and the chromogen was 3,3'-diaminobenzidine (DAB). Parts of the sections were counterstained with cresyl violet. Immunohistochemical control sections were handled in a similar manner, except that primary antibodies were omitted.

Transmission electron microscopic investigations

For electron microscopy, 80 μm thick sections were cut with a Vibratome and used for immune-electron microscopy using antibody against PV before flat embedding. For post-fixation, sections were first immersed in a solution of 2.5% glutaraldehyde diluted in PB and in a 1% solution of osmium tetroxide in PB. After flat embedding, areas of interest were cut using light microscopic control and reblocked in Durcupan resin. The same embedding procedure was performed on sections without immunohistochemistry. From the block embedded in synthetic resin, ultra-thin sections were made with an ultramicrotome, which were collected on single slot collodion-coated grids and then contrasted with uranyl acetate and lead citrate. Sections were examined in a JEOL 1200 EX-II and JEM-1400Flash transmission electron microscopes (TEM).

Quantification

The number of PV+ neurons in normal position (in the granule cell layer and in the hilus) and ectopic position (in the molecular layer and located along the hippocampi fissure), as well as the degree of axonal sprouting, were determined on light microscopic digital images using the iTEM program (Olympus). Then, the ratio of ectopic and normal cells was determined. We quantified the distribution of PV+ axon terminals on different target structures in immunoelectron microscopic preparations of four HS patients.

The data obtained during the quantification were compared with the clinical data of the patients. Student's t-test and Spearmann's correlation analysis were used to evaluate the results, and statistical significance was determined at $p \leq 0.05$.

RESULTS

Parvalbumin-immunoreactivity in the control dentate gyrus

The majority of PV+ neurons were located in the hilus, in the subgranular zone below the granule cell layer and inside the granule cell layer of the GD. PV+ axon terminals formed extensive network around somata of granule cells. Density of PV+ axons was higher in the inner half of the granule cell layer closer to the hilus than in the outer half of the layer. A few axonal branch might protrude into the outer half of the granule cell layer, but PV+ axonal branches were rare in the molecular layer of the controls. Although rarely, cell bodies of a few PV+ cells have been found in the molecular layer, close to the granule cells.

Parvalbumin-immunoreactivity in TLE patients

The pattern of PV-immunoreactivity in the DG of non- epileptic controls was similar to that of TLE patients with MCD, with tumor-induced epilepsy and with negative MRI results. On the other hand, many differences were seen in the GD of TLE patients with HS (and partly with dual pathology). PV+ cells almost completely disappeared from the subgranular position and from the hilus of the GD. Parts of the granule cell layer did not contain PV+ axons, while other parts showed strong PV-immunoreactivity. The whole width of the granule cell layer contained PV+ axon terminals. Frequently, PV+ axons ran perpendicularly to the granule cell layer, intruded deep into the molecular layer. Regarding the extent of PV+ axons' sprouting in the molecular layer of the DG, we have observed that significantly larger segments of the granule cell layer were covered with sprouted PV+ axons in HS patients and the extent of axon sprouting was also significantly greater in HS patients than in other patients' group. Our results clearly indicate the correlation between the presence of PV+ axons in the molecular layer of GD and the HS.

Ectopic PV-immunoreactive cells and sprouting of PV-immunoreactive axons

We analyzed the number of PV+ cells in normal location and in ectopic location in our TLE patients' groups. Regarding the number of PV+ cells in normal location, there was a significant difference between the control group and the HS group, as well as the group of patients with double pathology. Parallel with the decrease in normally-positioned PV+ cells, the number of ectopic PV+ neurons increased significantly in the HS group compared to control patients, and the ratio of ectopic / normally-positioned cells in the HS groups was significantly higher than in the groups with other etiologies.

Correlation analysis revealed significant assotiation between the extent of PV+ axon sprouting and the decrease in normally-located hilar and subgranular PV+ cells in HS group, as well as in the whole cohort's population. Significant correlation was found between the ratio of ectopic / normally-located PV+ cells and the extent of PV+ axon sprouting in HS patients and in the whole cohort population.

Correlation between PV-immunoreactive cells, axonal sprouting and clinical data of the patients

Correlation between the extent of PV+ axonal sprouting and the clinical data of the patients (e.g. duration of epilepsy, frequency of the seizures, gender ratio, side of HS, age at onset of TLE and history of febrile seizure), has been analyzed. Furthermore correlation between the

density of ectopic PV+ cells and the clinical data of the patients has also been analyzed. In the HS group, significant association was found between duration of the disease and the extent of PV+ axonal spouting, and significant negative correlation could be detected between duration of the disease and the density of normally located PV+ cells. Extent of PV+ axonal sprouting and the ratio of ectopic / normally-located PV+ neurons was considerably higher in those HS patients whose epilepsy started in childhood or in adolescence, but the difference was not statistically significant. Patients with childhood febrile seizure (FS) had larger extent of sprouting, than those that had not FS, furthermore the number of ectopic PV+ cells and the ratio of ectopic / normally-located PV+ neurons were higher than those that had no FS, although the difference was not statistically significant. Lack of statistical significance might be explained by the relatively low number of patients who experienced FS in childhood.

Synapses in the granule cell layer and in the molecular layer of the dentate gyrus

In tumor-related epilepsy, the number of symmetric synapses/soma of granule cells were similar to that found in controls. Compared to non-epileptic controls and tumor related TLE, the number of symmetric axo-somatic synapses/granule cells were decreased in HS. Symmetric synapses formed by PV+ axon terminals was observed on the somata of granule cells, on proximal dendrites, In addition, PV+ axon terminals formed symmetric synapses on small diameter distal dendrites and dendritic spines. We studied of the distribution of PV+ axon terminals on different target structures. We have found that the ratio of perisomatic synapses were significantly lower than that of the axo-dendritic synapses, and PV+ axon terminals formed significantly more synapses on dendritic spines than on somata of granule cells in those areas of the granule cell layer, where sporuting in the molecular layer was visible with light microscope.

DISCUSSIONS AND CONCLUSIONS

Morphological changes in PV-immunoreactive cells and axons in the hippocampus of epileptic patients

The main finding of the present study is that sprouting of axons of PV+ cells in the dentate molecular layer is associated with HS and with change of PV+ neurons' number and position in the DG. We have observed alteration in target structure of PV+ axon terminals in the DG. PV+ axon sprouting was significantly stronger in HS than in other TLE groups or in non-

epileptic controls. In addition, we have found significantly more ectopic PV-ir neurons in the dentate molecular layer and along the hippocampal fissure in HS than in the control group. Regarding the ratio of ectopic / normally-located PV+ cells, significantly larger ratio was observed in HS than in other TLE groups or in non-epileptic controls. Moreover, we have observed a significant correlation between the degree of PV+ sprouting and the ratio of ectopic / normally-located PV+ neurons.

Sprouting of PV-immunoreactive axons, presence of ectopic PV-immunoreactive neurons and clinical data of the patients

Loss of PV+ basket and axo-axonic cells have been detected only in human TLE associated with HS. The presence of ectopic PV+ neurons and sprouted PV+ axons in the molecular layer has never been described in epileptic animal models. A possible explanation can be that epilepsy in humans lasts several years compared to the relatively short duration of epileptic activity in experimental animals. The association between the duration of epilepsy and the sprouting of PV+ axons supports this argument.

PV+ sprouting frequently occurred in those HS patients whose TLE started in childhood and in adolescence. A previous study has shown that a substantial population of TLE patients with HS and with history of childhood FS had the onset of epilepsy during childhood or during adolescence, thus our work indicates the role of FS in sprouting of PV+ axons. We have found that patients with history of FS had stronger sprouting than those patients who had no FS. Similarly, the ratio of ectopic / normally-located PV+ cells was higher among patients with history of childhood FS comparing to those without FS, although, the difference was not statistically significant. Association between FS and dispersion of granule cells in the molecular layer of the GD in TLE has been previously reported. In addition, one may suppose that sprouted PV+ axons terminate on these dispersed granule cells, although our electron microscopic observations contradict this suggestion, since sprouted PV+ axon terminals terminated not only on somata and proximal dendrites, but on distal dendrites and dendritic spines.

Possible functional consequences of ectopic PV-immunoreactive cells and axon sprouting

The changes in the target structure of PV+ axon terminals in HS may result in changes of the excitation of granule cells. According to the dormant basket cell hypothesis, axo-somatic interneurons receive less excitatory input due to the vulnerability of mossy cells in the hilus. but mossy cells are not more vulnerable than other neurons (e.g. CA1, CA3 pyramidal cells) of

the human epileptic hippocampal formation. The activity of ectopic PV+ cells may strengthen inhibition on granule cells provided by normally-located basket and axo-axonic cells that support the theory of disinhibition.

Our data clearly indicate anatomical difference of internal circuitry of the DG in TLE with different etiologies. Our present data demonstrate axonal reorganization of PV+ neurons beyond their normal territory in the molecular layer of the DG and change in their target selectivity. These changes may contribute to the formation of aberrant neuronal circuitries of the DG that could support the epileptic condition.

EXAMINATION OF WHITE MATTER SYNAPSES IN HUMAN SAMPLES

MATERIALS AND METHODS

Clinical data

Surgically removed tissue of the middle temporal gyrus of pharmacotherapy-resistant TLE patients (n = 14) were used in this study. In 10 patients HS, in four patients MCD have been verified with magnetic resonance imaging (MRI). Neocortical white WM of temporal lobe tissues from non-epileptic patients with intracranial tumor (n = 3) and from autopsy (n = 3) were used as controls. Samples of TLE patients and of patients with intracranial tumor were obtained from the UPMS, while autopsy control samples were granted by the Institute of Experimental Medicine, Budapest. Tissue was obtained and used in a manner compliant with the Declaration of Helsinki. All procedures were approved by the regional and institutional committees (ETT TUKEB 15032/2019/EKU, PTE KK RIKEB/5342), and tissue samples were processed and histological evaluation has been carried out accordingly.

Tissue processing and immunohistochemistry

The cortical tissues resected from TLE and tumor patients were fixed in the same way as described in the previous work. Autopsy samples were stored following a deep-freezing procedure, and were fixed in 4% PFA buffered with PB (0.1 M, pH=7.4) immediately after melting. Tissue blocks containing neocortical WM and gray matter (GM) were embedded into paraffin and 10 μm thin sections were prepared. Following the removal of paraffin and antigen retrieval, immunohistochemistry was performed with a primary mouse monoclonal anti-SYN (Novocastra, New Castle upon Tyne, United Kingdom, 1:400) antibody. Other parts of tissue blocks of surgically removed samples of TLE patients were cut with a Vibratome at 80 μm, and free-floating sections were processed for immunohistochemistry. In this case the primary antibody was anti-NeuN antibody (Chemicon, Temecula, CA, USA, 1:500).

Transmission electron microscopy

The neocortical tissues of TLE patients were also prepared for immuno-electron microscopy, similarly to that described in previous chapter. The immunoreaction was also performed with a primary anti-SYN antibody (Novocastra, New Castle upon Tyne, 1:400), and the immunoreactive axon terminals were examined in Jeol 1200 EX-II and JEM-1400 Flash transmission electron microscopes (TEM).

Quantification

On light microscopic sections, the average number of WM neurons per 1 mm^2 area in the deep WM were determined using Neurolucida software (Neurolucida 2.0, Microbrightfield Inc., Williston, VT). The data of different patients were averaged and the density of NeuN-immunoreactive cells was expressed as neurons/mm^2 ± standard deviation (SD). The determination of the optical density of the SYN+ profiles was performed using Image J software (NIH, US-supported image analyzer) on digital pictures taken with light microscope. The density values measured of each patient's and control's samples were averaged. The density of SYN+ profiles were expressed as numerical values ± SD without measure unit.

Optical density of SYN-immunoreactivity in autopsy controls was compared to biopsy controls, and the difference between the SYN density measured in the controls and in TLE was also determined. We investigated a correlation between the optical density of SYN-immunoreactivity and the density of WM neurons, as well as clinical data of TLE patients. Student t-test and Spearman correlation analysis were used to evaluate the results. Statistical significance was set at $p \leq 0.05$.

Neuropsychological examinations

Preoperative verbal and visual memory performance of TLE patients were tested. Visual attention was assessed using the Corsi Block-Tapping task, visual construction ability and memory were assessed using the Rey–Osterrieth Complex Figure test. Verbal learning and memory were tested using the Hungarian version of the Rey auditory verbal learning test (AVLT) and the digit span task. The relationship between the cognitive data of TLE patients and the results of our histological observations was examined using linear regression analysis. Statistical significance was set at $p \leq 0.05$. Statistical analyses were performed by IBM SPSS software package (version 25. SPSS Inc, MN).

RESULTS

Synaptophysin-immunoreactivity in the white matter

In samples from TLE patients and control groups (biopsy and autopsy), SYN+ profiles could be seen as small dots in the sections. As it was expected, dense SYN-immunoreactivity was observed in the GM and the border between GM and WM was clearly outlined. In low numbers, SYN+ puncta could be also found in the WM of the control group. The border

between WM and GM appeared blurred in a few HS and MCD cases. The most striking observation in TLE samples was the higher density of SYN-immunoreactive puncta in the WM. The SYN+ profiles were also examined by TEM: SYN-immunoreactivity was located in the presynaptic axon terminals.

Quantification of synaptophysin-immunoreactivity in the white matter

Quantification of SYN-immunoreactivity was performed in the deep WM, approximately 500 μm below the border of WM and GM in both autopsy and biopsy controls, as well as in TLE samples. The average density of SYN immunostaining in the neocortical WM of 14 TLE patients was significantly higher than that in control samples. The difference between SYN-immunodensity in WM of the HS patients and that of controls were statistically significant.

Correlation between optical density of synaptophysin-immunoreactivity and density of neurons in the neocortical white matter

In case of 13 TLE samples, data on both optical density of SYN immunostaining and density of NeuN-immunoreactive WM neurons were available. Spearman's analysis has revealed significant positive correlation between SYN optical density and density of neurons in the WM. In addition, significant correlation was observed in the WM of HS patients between SYN-optical density and density of neurons. Due to the low number of patients in the MCD group, association between SYN optical density and density of WM neurons was not statistically significant. However, larger SYN optical density was detected in those samples that contained larger number of neurons per unit area.

Correlation between histological findings and the clinical and cognitive data of TLE patients

Correlation analysis revealed no significant association between the SYN-immunoreactivity in the neocortical WM and the following clinical parameters of the patients: age and gender of patients, age at onset of epilepsy, duration of the disease, frequency of the seizures and occurrence FS in childhood. Regarding the postoperative outcome of patients, the Engel classification was used. Twelve of our TLE patients became seizure-free and belonged to Engel class 1. One patient belonged to class 2, which indicates a slightly worse postsurgical outcome. No meaningful improvement occurred in one patient who belonged to class 4. Engel class 1 can be further divided into subclasses. We separately examined those patients who became completely seizure free without anti-epileptic drugs (class 1A) and other patients who

belonged to Engel class 1B-D. Regarding other Engel classes, subclasses were not considered. Our analyses revealed that post-surgical outcome significantly correlated with the optical density of SYN-immunoreactivity in the whole TLE population. In the subgroups of HS, in which the SYN-immunodensity was the highest, all patients became seizure free and belonged to Engel class 1. Examination of the density of WM NeuN+ cells showed a similar result.

Linear regression analysis between the histological findings and the cognitive performance of patients indicated that neuronal and synaptic densities in the WM were associated with verbal memory. The optical density of SYN-immunoreactivity was significantly correlated with the interference in AVLT. Regarding short-term verbal memory, a significant correlation was observed between scores of digit span forward test and SYN-immunodensity, as well as density of neurons. The scores of visual attention and memory tests did not show significant correlation with SYN-immunodensity and with the density of NeuN+ cells. Analyzing the association between WM neuronal density and verbal memory performance of HS patients, a significant linear regression was found between the density of NeuN+ cells and the scores of digit span forward test.

DISCUSSIONS AND CONCLUSIONS

We have shown that a significantly higher density of synapses could be found in the neocortical WM of TLE patients than in controls, and we have observed a significant correlation between the density of synapses and neurons in the WM of epilepsy patients. Density of synapses in the WM significantly correlated with the postsurgical outcome of TLE patients. In addition, we have found significant positive regression between the number of neurons, the density of synapses and the verbal memory performance of patients. In groups of TLE patients with HS and with MCD, synapse density was separately examined. The density of synapses was significantly higher in the WM of TLE patients with HS than in controls, and in HS a significant correlation could be found between the density of synapses and the density of neurons.

Neuronal numbers in the WM have been reported to be increased in epileptic patients compared to non-epileptic controls, but regarding the functional significance, as well as the synaptic connections of WM neurons, no clear information was available. Therefore, we have studied synapses that were visualized with immunohistochemistry based on the SYN protein content of the presynaptic axon terminals. SYN+ profiles have been observed in WM of both

TLE patients and control samples. Under the light microscope, SYN+ terminals were visible as small dots, but using immunoelectron microscopy, we verified the localization of SYN in presynaptic axon terminals.

Neurons and synapses in the white matter

The impact of WM neurons on generation and maintenance of seizure activity has not yet been proven. In TLE patients we revealed a significant positive linear correlation between the density of WM neurons and the optical density of SYN-immunoreactivity, which suggests that WM neurons may be functionally active, integral parts of the neuronal circuitries in the temporal lobe in TLE. The SYN+ terminals might originate form cortical and/or subcortical neurons, but the WM neurons might be the source of SYN+ presynaptic terminals as well. Correlation of the neuronal numbers and the optical density of SYN-immunoreactivity with neuropsychological data of patients - including the values related to verbal memory - revealed a significant positive correlation, and a significant regression was observed between the optical density of SYN-immunoreactivity and interference in AVLT. These data clearly indicate the functional importance of WM synapses in the temporal lobe.

The existence of functional neural networks within the WM is supported by MRI studies. Using fMRI, results indicated functional disruption in WM networks in mesial temporal sclerosis.

The significance of synapses in the WM of the temporal neocortex is highlighted by the correlation of optical density of SYN-immunoreactivity and the postsurgical outcome of TLE patients. We observed significant correlation between postsurgical outcome of TLE patients and the optical density of SYN-immunoreactivity showing that the larger optical density of SYN-immunoreactivity, the better is the postsurgical outcome of the patients. This association indicate that WM synapses might play a substantial role in the generation and maintenance of epileptic seizures in patients, and the removal of the area containing WM neurons and synapses largely contributed to the favorable postsurgical outcome of the patients.

The exact way and the time of formation of WM synapses on WM neurons are still unclear, however, two basic explanations may occur. It may be due to abnormal cortical development, which is supported by our results (increased number of neurons and its correlation with the density of synapses). The increased SYN density in the WM found in our study suggests that WM neurons receive synaptic input, and the origin of these axons could be the cortical neurons in normal position as well as WM neurons. WM neurons may terminate on each other and form abnormal subcortical circuitries which may play a role in the development and maintenance of

epilepsy. Another explanation for the high density of WM synapses can be synaptic reorganization that is a known feature of epilepsy.

Our work indicates the functional importance of WM neurons and synapses, although, further research is needed about the exact role of them in TLE. The presence of functional networks within the WM may open new avenues of research in cognitive and clinical neuroscience.

SUMMARY

Summary of findings in the dentate gyrus of epileptic patients

1. In HS, the ratio of ectopic / normally-located PV+ cells and the degree of PV+ axon sprouting are significantly higher than in the TLE groups with other etiologies.
2. In HS, we found a significant positive correlation between the degree of axon sprouting and the ratio of ectopic / normally-located PV+ cells.
3. Based on our results obtained by immuno-electron microscopy, we can conclude that in the areas where axon sprouting was visible by light microscopy, the postsynaptic target structure of PV+ cells partially changes in HS: axon terminals terminate on the distal dendrites or dendritic spines of granule cells, rather than on their somata.
4. In HS, as a result of synaptic reorganization, the axons of PV+ neurons are also found in the molecular layer of the GD, and their target selectivity changes.
5. These changes may contribute to the formation of aberrant neuronal circuitries in the GD and thus support the development and maintenance of epilepsy.

Summary of results found in the neocortical white matter of epileptic patients

1. We found that the optical density of SYN-immunoreactivity is significantly higher in TLE patients than in controls.
2. We found a correlation between the number of WM neurons and the optical density of SYN-immunoreactivity.
3. We found a significant correlation between the postoperative outcome of TLE patients and the SYN density.
4. We also found a correlation between the histological results of TLE patients and verbal memory. Short-term verbal memory showed a significant positive correlation with the number of WM neurons and the optical density of SYN-immunoreactivity.
5. Our results suggest that the WM neurons found in TLE patients are functionally active, as they receive a large number of synaptic inputs from other cortical, subcortical or WM neurons, and we assume that they contribute to epileptic neuronal networks.

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