Epilepsy & Behavior 25 (2012) 110–119

Contents lists available at SciVerse ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Correlation between calbindin expression in granule cells of the resected hippocampal dentate gyrus and verbal memory in temporal lobe epilepsy

Kázmér Karádi [a], József Janszky [b,c], Csilla Gyimesi [b,c], Zsolt Horváth [d], Tivadar Lucza [a], Tamás Dóczi [c,d], János Kállai [a], Hajnalka Ábrahám [e,*]

[a] Department of Behavioral Sciences, Faculty of Medicine, University of Pécs, Szigeti u. 12., Pécs 7624, Hungary
[b] Department of Clinical Neurophysiology and Epileptology, Neurology Clinic, University of Pécs, Rét u. 2., Pécs 7623, Hungary
[c] Neuroscience MR Research Group, Hungarian Academy of Science, Rét u. 2., Pécs 7623, Hungary
[d] Department of Neurosurgery, Faculty of Medicine, University of Pécs, Rét u. 2., Pécs 7623, Hungary
[e] Central Electron Microscopic Laboratory, Faculty of Medicine, University of Pécs, Szigeti u. 12., Pécs 7624, Hungary

ARTICLE INFO

Article history:
Received 10 February 2012
Revised 5 June 2012
Accepted 7 June 2012
Available online 15 July 2012

Keywords:
Hippocampal sclerosis
Calcium-binding protein
Tumor
Cortical malformation
Learning
Temporal lobe epilepsy

ABSTRACT

Calbindin expression of granule cells of the dentate gyrus is decreased in temporal lobe epilepsy (TLE) regardless of its etiology. In this study, we examined the relation between reduction of calbindin immunoreactivity and the verbal and visuo-spatial memory function of patients with TLE of different etiologies. Significant linear correlation was shown between calbindin expression and short-term and long-term percent retention and retroactive interference in auditory verbal learning test (AVLT) of patients including those with hippocampal sclerosis. In addition, we found significant linear regression between calbindin expression and short-term and long-term percent retention of AVLT in patients whose epilepsy was caused by malformation of cortical development or tumor and when no hippocampal sclerosis and substantial neuronal loss were detected. Together with the role of calbindin in memory established in previous studies on calbindin knock-out mice, our results suggest that reduction of calbindin expression may contribute to memory impairments of patients with TLE, particularly, when neuronal loss is not significant.

1. Introduction

Temporal lobe epilepsy (TLE) is the most common form of intractable focal epilepsies with complex partial seizures. In many patients (48–75%), therapy-resistant TLE is associated with sclerosis of the medial temporal lobe structures [1,2]. Less frequently, TLE is related to malformation of cortical development (MCD; 13–45%) and tumors (27–33%) of the central nervous system [3]. Mesial temporal sclerosis includes hippocampal sclerosis (HS) with severe pyramidal cell loss in CA1 and CA3 regions of Ammon's horn and subiculum as well as loss of hilar mossy cells in the dentate gyrus [4–7]. In addition to neuronal cell death, reorganization of synaptic circuits, including sprouting of mossy fibers and axons of other hippocampal neurons, is a common finding in both human TLE and experimental TLE [8–15]. In contrast to mesial temporal sclerosis, neurons of the hippocampal formation are mostly preserved in MCD and in tumor-related epilepsy (TUE) when the tumor does not invade the hippocampal formation [16–19]. However, in lesion-induced TLE, including TUE, a few previous studies suggested the involvement of the hippocampal formation in the epileptogenesis, even in cases when the epileptogenic focus was located outside the hippocampal formation and when no pathological alteration was visible in the hippocampus [20–23]. Recently, we have shown a common histological alteration in the hippocampal dentate gyrus in all forms of TLE regardless of the etiology of seizures [19]. Despite the absence of considerable neuronal death, granule cells of the dentate gyrus revealed diminished calcium-binding protein calbindin-D28k (CB) immunoreactivity in MCD and TUE, similar to what was observed earlier in HS [19,24]. In addition, the decrease of CB expression in the granule cells correlated negatively with the age of epilepsy onset and correlated positively with the duration of epilepsy [19].

Calbindin acts as a calcium-ion sensor and buffer, and it is a potential eliminator of intracellular calcium in cases when the cell is overloaded, e.g. in neuronal injuries. However, the role of CB in neuronal cell death in TLE is unclear; both neuroprotective and deleterious effects have been reported [25–28]. In knock-out mice lacking functional CB, neuronal loss following ischemia was not more severe than in wild-type controls. In contrast, reduced level of CB protein causes impairment of memory formation in experimental animals [29]. Results from CB-deficient mice indicated that CB plays an important role in long-term potentiation (LTP) and synaptic consolidation of hippocampal memory [29,30]. Further support for the possible role of CB in memory formation is that expression of CB by the granule cells and their axons during postnatal development correlates with the functional development of the dentate

* Corresponding author at: Central Electron Microscopic Laboratory, Faculty of Medicine, University of Pécs, Pécs 7602, P.O. Box 99, Hungary. Fax: +36 72 536 001x1510.
E-mail address: hajnalka.abraham@aok.pte.hu (H. Ábrahám).

1525-5050/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.yebeh.2012.06.007

gyrus and behavioral maturation of rats [31,32]. Studies in humans show that morphological maturation of granule cells as indicated by their CB immunoreactivity is a long-lasting event, and it comes to an end by the time children are able to solve hippocampal-dependent memory tasks similar to spatial navigation tasks used in rodent studies [33,34].

In addition to spatial memory, the hippocampal formation plays an important role in declarative memory, which is suitable for intentional recall of learned information of facts and events [35,36]. In patients with chronic TLE, impairment of declarative memory has been demonstrated, and epilepsy severely affects long-term delayed recall of visual and verbal information [37,38]. Some patients with a focus in the left temporal lobe could not recall autobiographical memories from their childhood [39]. Left HS correlated with impairments in verbal episodic memory while visuo-spatial working memory was affected in right HS [40,41]. In addition, deficiency of verbal memory in left HS and deficiency of visual memory in right HS were observed in patients with early-onset, long-term duration and high-frequency of seizures [42]. Proton magnetic resonance spectroscopy (^{1}H MRS), which is assumed to be suitable for providing information about neuronal loss and gliosis, revealed correlation between ^{1}H MRS values and verbal memory scores [43]. Postoperative histological studies showed that pyramidal cell loss in the removed hippocampal formation significantly correlated with preoperatively detected memory impairments of patients [44–49]. Loss of hilar neurons and granule cells as well as lower proliferative capacity of neuronal precursor stem cells of the subgranular layer of the dentate gyrus was correlated with impaired memory function [44,45,47,49,50].

The granule cells of the dentate gyrus are stimulated by entorhinal excitatory afferents and form the postsynaptic site of the first synapse of the trisynaptic hippocampal circuit; therefore, they are essential for memory formation. Moreover, granule cells express CB, a calcium-binding protein that is critical in hippocampal learning processes [29,30]. In our present study, we analyzed the possible correlation between the decrease of CB expression in the granule cells of surgically resected hippocampal formations and different presurgical learning and memory functions of patients with drug-resistant TLE.

2. Methods

2.1. Patients

Surgically removed tissues of the hippocampal formation of adult patients with medically-refractory TLE (n = 17) were used. In this study, we only included those patients who underwent extensive psychological testing before the operation. Ten of these patients had HS verified by MRI. In five patients, epilepsy was attributed to MCD, and in three of these patients, no pathological alterations could be detected in the hippocampal formation with MRI. Two patients suffered from epilepsy caused by tumor located in the temporal lobe, but the hippocampal formation was not invaded. The same cohort of epilepsy patients was used in our previous study describing the relationship between CB loss and etiology, but behavioral studies were not included [19]. Demographic data and clinical findings of the patients are listed in Table 1.

Epilepsy patients were evaluated in the Department of Epileptology, Neurology Clinic at the University of Pécs. Video/EEG monitoring was performed on all patients, and the temporal epileptic focus was identified. All patients underwent high-resolution brain MR imaging performed on a 3-T MR machine (Siemens Trio, Siemens AG, Erlangen, Germany) using a special protocol for TLE focusing on the temporal lobe and especially on the hippocampal formation.

2.2. Neuropsychological testing

Neuropsychological assessment included IQ, verbal intelligence quotient (VQ) and performance quotient (PQ) testing using the Wechsler Adult Intelligence Scale. Verbal skills and naming ability were tested using the verbal fluency test and Boston Naming test. In the phonemic version of the verbal fluency test, the subjects had to produce words beginning with the letters F, A, and S in one minute. In the categorical version of the verbal fluency test, the subjects produced animals in one minute. In the Boston Naming test, the subjects named pictures of 60 line-drawing objects. Verbal attention was measured with the forward version of the digit span task. Visual attention was assessed using the Trail Making test and the forward version of the Corsi Block-Tapping task [51]. Visual construction ability and memory were assessed using the Rey–Osterrieth Complex Figure (ROCF) test. After copying the ROCF, the patient had to draw it from memory in delayed recall (30 min). In the ROCF test, a standard Taylor's scoring system was administered with a maximum of 36 points over copying and memory versions. Each figure was divided into 18 different blocks. When the subject drew properly placed, correct blocks, 2 points were given. Properly placed and distorted or poorly placed and correct blocks were rated with 1 point. Distorted, poorly placed blocks were scored with half point. In case of absent or unrecognizable blocks, no points were given [52]. Verbal learning and memory were tested using a Hungarian version of the Rey auditory verbal learning test (AVLT). Auditory verbal learning test measures

Table 1
Summary of the clinical data of the patients with TLE.

Case no.	Age (Y)	Gender	Age of onset (Y)	Duration (Y)	Seizure frequency	Side of focus	MRI diagnosis
1	48	F	2	46	1–2/mo	L	HS
2	42	F	6	36	10–16/mo	R	HS
3	24	F	14	10	4–5/mo	R	HS
4	32	M	24	8	1/mo	R	HS
5	45	M	6	39	15/mo	L	HS
6	43	F	5	38	4–5/mo	L	HS
7	46	F	8	38	1–3/mo	L	HS
8	47	M	1	46	1–2/mo	L	HS
9	40	M	2	38	6–10/mo	R	HS
10	52	M	18	34	1–2/mo	R	HS
11	28	M	12	16	8–10/mo	R	Cortical heterotopia; HF is MRI negative
12	34	M	7	27	4–8/mo	R	Cortical dysgenesis
13	50	F	28	22	3–4/mo	R	Cortex and HF are MRI negative
14	31	F	12	19	No data	R	Cortex and HF are MRI negative
15	17	F	10	7	2–3/mo	R	Cortical and hippocampal dysgenesis
16	28	M	13	15	1/year	R	Tumor
17	27	M	18	9	No data	R	Tumor

Abbreviations: Y, years; mo, months; MCD, malformation of cortical development; R, right; L, left; HS, hippocampal sclerosis; M, male; F, female; TLE, temporal lobe epilepsy; MRI, magnetic resonance imaging; HF, hippocampal formation.

verbal learning ability using 15 common nouns (A and B lists). Five presentations of the A list were given. After each presentation, the subject had to recall the words from the list. Learning over five trials was evaluated. After the 5th trial, the B list was read, and the subject had to remember this new list. In the 7th trial, the subject recalled the A list without auditory presentation. The 8th trial presented the delayed recall after 20 min. After testing, we evaluated the total learning score (TL) by the total number of learned words over the first five trials, short-term retention on the 7th trial as a proportion of the 5th trial, and long-term retention on the 8th trial as a proportion of the 5th trial. Learning over trials was evaluated using the equation of $TL-(5\times \text{Trial } 1)$. Retroactive interference (RI) was calculated using the equation of $((\text{Trial } 5-\text{Trial } 7)/\text{Trial } 5)\times 100$ [53,54].

2.3. Surgical procedure

All surgeries were performed in the Department of Neurosurgery at the University of Pécs. In patients with HS, a lateral cortical resection included 3-4 cm of tissue from the middle temporal gyrus and 4–5 cm of the inferior temporal gyrus but spared the superior temporal gyrus on the left side and, in most cases, on the right too when that side was operated. A subpial aspiration of the uncus was performed superior to the level of the entorhinal sulcus, limen insulae, and anterior to the M1 segment of the middle cerebral artery. The ventricle was opened and retracted. The operative microscope was then used to partially remove the amygdala followed by the pes and head of the hippocampus. The hippocampus was resected to the level of the ambient cistern, but the mesial pia was not violated. The body of the hippocampus was removed separately back to the posterior bend. In patients with MCD or tumor, in addition to the hippocampal formation, resection included areas of the temporal lobe occupied by the tumor or the developmental malformation. Hippocampi of five non-epilepsy patients with tumors in the temporal neocortex were used as controls for immunohistochemistry. The temporal lobes with tumors invading the temporal neocortex but without epileptic seizures were surgically removed. Lobectomy was necessary to ensure removal of the tumor-infiltrated tissue that was in the vicinity of the hippocampal formation. In all five cases, histopathological examination confirmed that the hippocampi were not invaded by the tumor cells.

All procedures including the neuropsychological testing and surgery were carried out with the adequate understanding and written consent of each patient, were conducted in accordance with the Declaration of Helsinki, and were approved by the ethics committee of the University of Pécs.

2.4. Tissue processing

Resected hippocampal tissues of the patients were immediately immersed in 4% paraformaldehyde buffered with phosphate buffer (0.1 M PB, pH 7.4) and kept for approximately 12 h at room temperature under continuous agitation. Following fixation, 10-mm-thick blocks perpendicular to the septo-temporal axis of the hippocampal formation were cut, and the blocks were sectioned with a vibratome at 80 μm. Individual serial free-floating sections were collected and processed for immunohistochemistry.

An additional tissue block containing the hippocampal formation was dehydrated, embedded in paraffin and cut with a sliding microtome at 10 μm.

2.5. Immunohistochemistry

2.5.1. Immunohistochemistry on free-floating sections

After washing in Tris buffer (TB, pH 7.4) for three times at 10 min each, the sections were pretreated with a solution of 1% hydrogen peroxide for 30 min to block endogenous peroxidase followed by pre-incubation in 1% normal horse serum in TB containing 0.4% Triton X-100 (Sigma-Aldrich, Hungary) for 1 h. This step was followed by incubation with primary monoclonal mouse anti-CB antibody (1:5000; Swant, Bellinzona, Switzerland) for 72 h at 4 °C. Additional sections that underwent the same pretreatment were incubated with primary monoclonal mouse antibody against a neuronal specific marker NeuN (1:500; Chemicon, Temecula, CA). Binding sites of the primary antibodies were visualized with biotinylated secondary anti-mouse antibody (1:100; 4 h at room temperature) and the avidin–biotin–peroxidase detection system (1:50; Universal Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA). The chromogen used was 3,3′-diaminobenzidine (DAB, 0.04%). The tissue sections were then mounted on glass slides and air-dried. Sections stained with NeuN immunostaining were ethanol series-dehydrated, cleared with xylene, and cover-slipped with DePeX (Fluka, Switzerland). Sections with CB immunoreactivity were counterstained with cresyl violet then dehydrated, cleared, and cover-slipped. Immunohistochemical control sections were handled in a similar manner except that the primary antibody was omitted.

2.5.2. Immunohistochemistry on paraffin-embedded sections

Following deparaffinization and rehydration of the sections, microwave antigen retrieval was performed as described previously [55,56]. Briefly, following washes with TB three times for 10 min, the slides were placed in 80-ml plastic jars filled with citrate buffer (pH 6.0) and heated in a microwave oven (Optiquick Compact, Moulinex) operating at a frequency of 2.45 GHz and 800 W power setting. After three heating cycles of 5 min each, the slides were allowed to cool at room temperature and were repeatedly washed in TB.

For visualization of immunoreactive profiles under the light microscope, after washing, the sections were preincubated in blocking normal horse serum (1% in TB; Vector Laboratories, Burlingame, CA) for 1 h. This step was followed by overnight incubation with the primary mouse monoclonal synaptophysin (SYN) antibody (1:400; Novocastra, Newcastle upon Tyne, UK) in a humidified chamber at room temperature. After washing three times for 10 min, binding sites were visualized with biotinylated secondary antibody (1:100; 2 h at room temperature) and with the avidin–biotin–peroxidase detection system (1:50; Universal Vectastain ABC Elite Kit, Vector Laboratories, Burlingame, CA). Using chromogene DAB (0.04%) and peroxidase substrate hydrogen peroxide (0.003%) diluted in TB, the immunoreaction was carried out under visual control, monitoring progress via a light microscope and stopped by the removal of the DAB followed by washes in TB. The sections were then counterstained with cresyl violet, dehydrated in ascending concentration of ethanol, cleared with xylene and covered with DePeX. Control sections were treated similarly except that the primary antiserum was omitted from the procedure.

2.6. Quantification

The granule cells of the dentate gyrus were quantified using a Nikon (Optiphot) microscope attached to a computer running Neurolucida software (Neurolucida 2.0, MicroBrightField Inc., Williston, VT). Calbindin-immunopositive and CB-negative granule cells were counted in both the inner and outer limbs of the granule cell layer including those cells that were dispersed in the molecular layer of the dentate gyrus. The proportion of CB-positive granule cells was expressed as a percentage of the entire granule cell population counted, and the standard deviation was determined. The number of counted immunostained non-consecutive sections/patient containing the entire granule cell layer varied from 3 to 9. All granule cells of the available sections were counted using the entire depth of the preparation, and the numbers of granule cells counted per section varied between 1601 and 4720, depending on the size of the dentate granule cell layer. No differences of the antibody penetration were observed throughout the thickness of the sections.

Averages of the rate of CB-expressing granule cells were also calculated. Subjects whose value was below the average were classified as low-level CB group, while those above the average belonged to the high-level CB group.

2.7. Statistical analysis

Linear regression analysis and Spearman's correlation were performed to study the relation of preservation of CB immunoreactivity in the granule cells of the dentate gyrus and the patients' performance on the neuropsychological tests. In addition, the relations between the performance on the neuropsychological tests and the various parameters, such as the age of onset of epilepsy and frequency of seizures, were evaluated.

3. Results

3.1. General histopathology

In agreement with our recently published results [19] in patients with HS, cytoarchitectonics of the hippocampal formation visualized with NeuN immunostaining revealed extensive loss of pyramidal cells in the CA1 area (Sommer's sector) and neurons of the hilus and CA3c region of Ammon's horn. Granule cell pathology was found in all HS patients, and eight out of the ten patients had dispersed granule cells in the molecular layer. Bilaminar appearance of the granule cell layer was observed in two patients with HS. Moderate thinning of the granule cell layer and small cell-free gaps could be observed in three patients, respectively, indicating mild loss of granule cells in HS.

In contrast to the HS, no substantial neuronal loss was observed either in the CA1 or CA3 pyramidal layers or in the hilus of the dentate gyrus in MCD-related TLE as well as in MRI-negative cases and TUE. In one patient who suffered MCD-related epilepsy, mild end-folium sclerosis could be observed. In three patients, including the one with mild loss of hilar neurons, bulbous expansions of the CA1 pyramidal layer were visible, resulting in tectonic malformation of layers of Ammon's horn as well as the dentate gyrus, as described earlier [19,57]. In one patient of the MCD group, dispersion of the granule cells and, in another one, small gaps in the granule cell layer indicated mild granule cell pathology. In contrast to the HS group, in which only 20% of the patients had ectopic hilar granule cells, all the patients with MCD and those with negative MRI findings had relatively large numbers of ectopic granule cells. In patients with TUE, no hippocampal or granule cell pathology could be observed.

Synaptophysin immunoreactivity revealed sprouting of mossy fibers in 90% of the patients with HS, while no sprouting was observed in the dentate gyrus of patients with MCD- and tumor-related TLE or in MRI-negative cases (Fig. 1).

Pathological findings of the Ammon's horn according to classification described by Wyler et al. [58], Thom et al. [59] and Blümcke et al. [60] and that of the dentate gyrus according to the grading given by Blümcke et al. [61] are summarized in Table 2. In addition, Table 2 contains pathological findings in the adjacent, surgically removed temporal neocortex according to the classification of the International League Against Epilepsy, as well as the diagnosis of the brain tumors [62].

3.2. CB immunoreactivity in the granule cells of the dentate gyrus

Control hippocampal formations revealed strong CB immunoreactivity in the dentate gyrus. Somata of the granule cells of the entire granule cell population and their dendrites in the molecular layer of the dentate gyrus as well as their axons running and terminating in

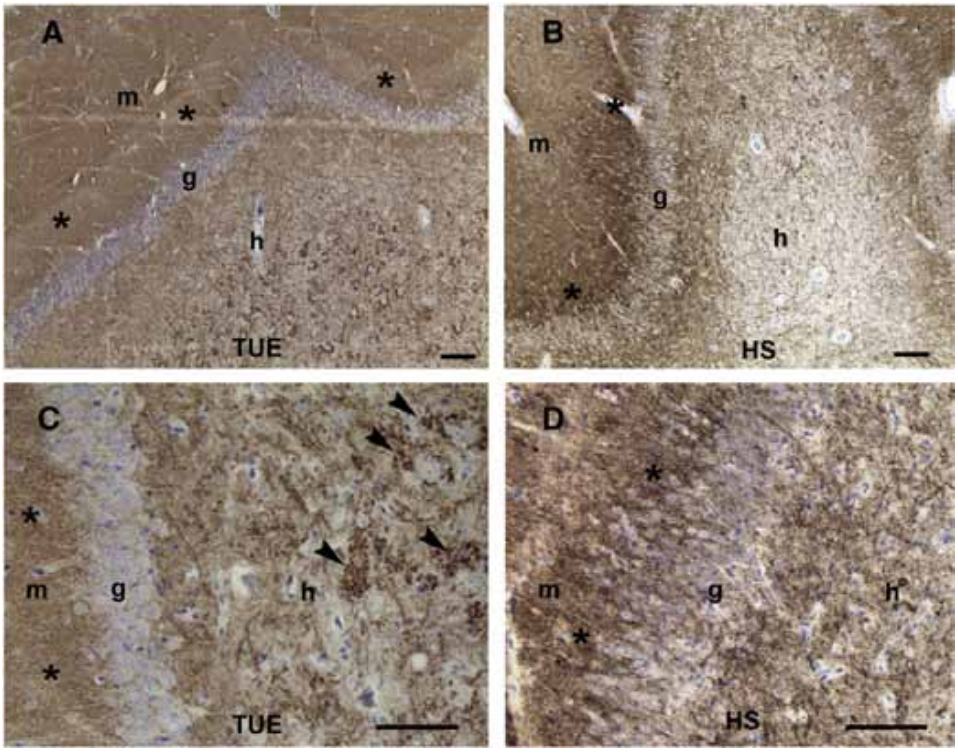

Fig. 1. Photomicrograph showing sprouting of mossy fibers (B and D) of the dentate gyrus visualized with synaptophysin immunohistochemistry. A: In the dentate gyrus of a patient with epilepsy caused by tumor, synaptophysin immunoreactivity reveals a light staining (asterisks) in the stratum moleculare (m) above the granule cell layer (g). B: In a patient with hippocampal sclerosis (HS), intense synaptophysin immunostaining (asterisks) is visible above the granule cell layer (g). C: Synaptophysin immunoreaction reveals large, intensely stained axon terminals of mossy fibers (arrowheads) in the hilus (h) of the dentate gyrus. D: Intense synaptophysin immunostaining (asterisks) in the molecular layer (m) above the granule cells (g) and the lack of large mossy fiber terminals in the hilus (h) of the dentate gyrus in a patient with HS. Scale bars: 100 μm.

Table 2
Summary of the histopathological data of the patients used in the study.

Case no.	Rate of CB expression	Degree of hippocampal pathology				Granule cell pathology							FCD or tumor diagnosis
		Wyler score	Thom score	Blümcke score	Other	Blümcke score	Layer thinning	Gaps	Dispersion	Bilaminar	Hilar ectopic granule cells	Sprouting	
1	38	3	CHS	MTS type 1a	–	GCP-type 1/2	+	–	+	–	–	+	NA
2	2.25	3	CHS	MTS type 1a	–	GCP-type 1	–	+	–	–	+	+++	FCD type IIIa*
3	16	3	CHS	MTS type 1a	–	GCP-type 2	–	–	++	–	–	+	FCD type IIIa*
4	39	2	CA1p	MTS type 2	–	GCP-type 1/2	–	+	+	–	–	+	No FCD
5	18	2	CA1p	MTS type 2	–	GCP-type 1/2	+	–	+	–	–	+	No FCD
6	11	3	CHS	MTS type 1a	–	GCP-type 2	–	–	+	+	–	++	FCD type IIIa*
7	6	3	CHS	MTS type 1a	–	GCP-type 2	–	–	+	–	–	++	FCD type IIIa*
8	17.6	2	CA1p	MTS type 2	–	GCP-type 2	–	–	+	–	–	++	No FCD
9	11.3	2	CA1p	MTS type 2	–	GCP-type 1/2	+	–	+	–	+	NA	FCD type IIIa*
10	17.3	3	CHS	MTS type 1a	–	GCP-type 1/2	–	+	–	+	–	+	FCD type IIIa*
11	10.4	1	Normal	No MTS	TM	No GCP	–	–	–	–	++	NA	NA
12	29.3	1	Mild EFS	Mild MTS type 3	TM	GCP-type 2	–	–	++	–	++	NA	FCD type Ic**
13	12.87	0	Normal	No MTS	–	No GCP	–	–	–	–	+++	–	FCD type Ic***
14	19.57	0	Normal	No MTS	TM	GCP-type 1	–	+	–	–	++	–	FCD type Ia****
15	16.37	0	Normal	No MTS	–	No GCP	–	–	–	–	+++	–	FCD type Ia****
16	40.2	0	Normal	No MTS	–	No GCP	–	–	–	–	–	–	Intracranial cholesteatoma
17	20	0	Normal	No MTS	–	No GCP	–	–	–	–	+	–	Astrocytoma (WHO grade I)

Abbreviations: CB, calbindin; CHS; classical hippocampus sclerosis; CA1p, partial CA1 loss; EFS, end-folium sclerosis; MTS, mesial temporal sclerosis; TM, tectonic malformation; GCP, granule cell pathology; FCD, focal cortical dysplasia. FCD type IIIa*, FCD type IIIa with heterotopic neurons in the cortical white matter; FCD type Ic**, abnormal cortical lamination in the subicular complex; FCD type Ic***, abnormal cortical lamination in the transentorhinal cortex and lentiform heterotopic neuronal groups in the white matter; FCD type Ia****, FCD type Ia with blurred boundary between white and gray matter.

the hilus of the dentate gyrus and along the CA3 pyramidal layer were CB immunoreactive (Figs. 2A and E). In contrast, substantial reduction of CB immunoreactivity was observed in each case of epilepsy including cases of HS, MCD and TUE (Figs. 2B–D, F–H, Table 2), and approximately only one-fifth ($19.06 \pm 11.26\%$) of the entire granule cell population expressed CB. The rate of reduction of CB expression, however, was variable among patients, and the proportion of CB-immunoreactive granule cells relative to the entire granule cell population ranged between 2.25% and 40.2%. Slight differences could also be observed between patient groups. Patients with HS were noted to have great reduction of CB immunoreactivity with an average of 17.63% CB-immunoreactive granule cells. Similar strong reduction of CB-expressing granule cell numbers was detected in MCD, and only 17.5% of the cells of the granule cell layer were CB positive. In the TUE group, one-third of the granule cells of the entire granule cell population expressed CB (mean: 30.1%). Since we had patients with a focus in the left as well as in the right temporal lobe, we compared CB expression in the left and in the right dentate gyri. The average percentage of the CB-immunoreactive granule cells among all the granule cells in the left dentate gyrus was $18.12 \pm 10.89\%$ while it was $20.29 \pm 11.00\%$ in the right one. Therefore, no substantial difference could be detected in CB immunoreactivity of dentate granule cells of the right and left hippocampal formations.

3.3. Correlation between CB immunoreactivity and patients' neuropsychological performance

Averaged scores of the neuropsychological data of the patients are summarized in Table 3. Onset of epilepsy did not show correlation between test scores. Significant negative correlation was observed between the frequency of seizures and CB expression ($r = -0.56$, $p<0.05$), and scores of IQ ($r = -0.86$, $p<0.05$), VQ ($r = -0.72$, $p<0.05$) and PQ ($r = -0.81$, $p<0.05$). Duration of epilepsy had a negative correlation with the results of the Boston Naming test ($r = -0.72$, $p<0.05$).

Linear regression was calculated between averaged scores of the verbal and visual memory tests and the rate of CB expression. Significant linear correlation was found between expression of CB and scores of short-term retention ($F(1,14) = 5.98$; $p<0.05$; Fig. 3), long-term retention ($F(1,14) = 6.18$; $p<0.05$; Fig. 4), and retroactive interference of AVLT ($F(1,14) = 6.03$; $p<0.05$; Fig. 5) of all patients, regardless of the epilepsy etiology.

Subjects whose dentate gyrus contained less than $19.06 \pm 11.26\%$ CB-immunoreactive granule cells relative to the entire granule cell population belonged to the group classified as the low-level CB group, while those with values above the mean represented the high-level CB group. A one-way ANOVA test showed significant differences when scores of short-term retention ($F(1,15) = 8.08$; $p<0.05$), long-term retention ($F(1,15) = 7.15$; $p<0.05$), and retroactive interference ($F(1,15) = 8.02$; $p<0.05$) of AVLT of patients that belong to low-level CB group were compared to the high-level CB group. Scores of short-term and long-term retention were higher and that of retroactive interference was lower in the high-level than in the low-level CB group (Table 4). Repeated measures ANOVA did not show a significant difference between low-level and high-level CB groups during the learning period (first five trials) ($F(1,4) = 0.322$, n.s.). The largest difference between the two groups was found after the learning period of the first five trials ($F(1,4) = 30.31$, $p<0.0001$). These results indicate that the decrease of CB expression in the granule cells of the dentate gyrus negatively influences verbal memory scores in patients with TLE.

However, in these analyses, patients with HS were also involved. The severe neuronal loss, typical for HS, may also be responsible for memory impairments, as shown earlier [44–49]. Therefore, patients were separated into two groups by the etiology and MRI findings. A group was formed by patients with HS (group I), whereas patients with TLE caused by MCD, tumor and the MRI-negative cases belonged to group II. Statistical analysis showed positive linear regression between CB expression of patients of group II and scores of short-term ($F(1,5) = 13.76$, $p<0.05$; Fig. 6) and long-term percent retention of AVLT ($F(1,5) = 15.28$, $p<0.05$; Fig. 7). In contrast, rate of CB immunoreactivity in patients with HS (group I) did not correlate with either short-term ($F(1,8) = 0.93$, n.s.) or long-term percent retention of AVLT ($F(1,8) = 0.93$, n.s.).

Regarding visuo-spatial memory, no significant linear regression was seen between CB expression and performance on visual memory tests. We could not show significant differences between the low- and high-level CB groups in the visuo-construction part of the ROCF test ($F(1,15) = 34$), the visual memory part of the ROCF test ($F(1,15) = 1.8$), Trail Making A ($F(1,15) = 0.007$) and B versions

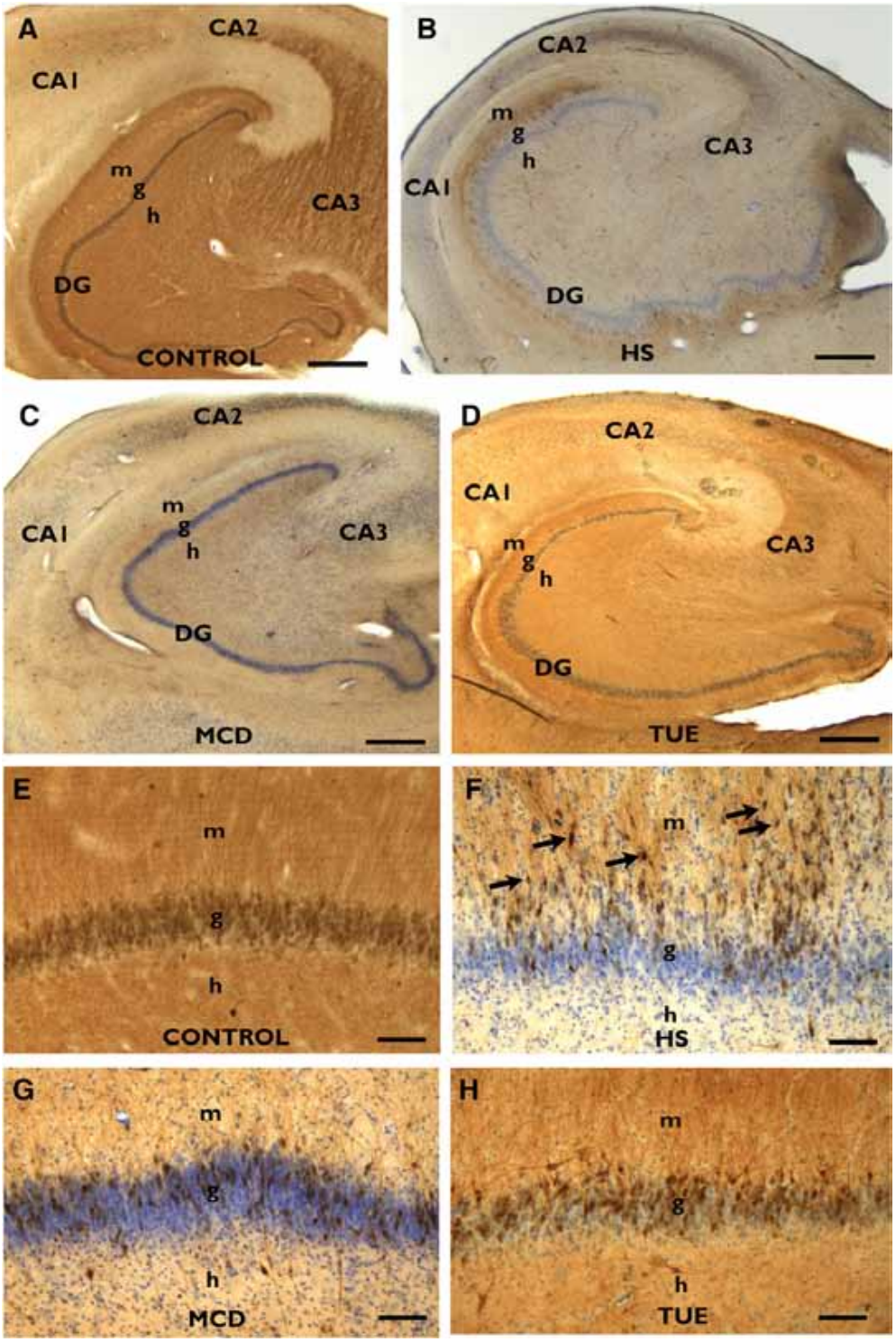

Fig. 2. Photomicrograph showing CB expression in the dentate gyrus (DG) of the hippocampal formation in controls (A and E) and in patients with TLE due to HS (B and F), cortical developmental malformation (MCD, C and G) and tumor in the temporal lobe (TUE, D and H). A: In controls, the entire granule cell population including the somata in the granule cell layer (g), dendrites in the molecular layer (m) and axons in the hilus (h) and by the CA3 pyramidal layer express CB. B: Large reduction of CB immunoreactivity in a patient with HS. C: Substantial decrease in CB immunopositivity in the dentate gyrus (DG) of a patient with MCD. D: In TUE, CB immunoreactivity was decreased. E: Somata of the granule cells in the granular layer (g) as well as dendrites in the molecular layer (m) and axons in the hilus (h) is strongly CB positive. F: Substantial reduction of CB immunoreactivity in the granule cells in a patient with HS. Arrows point to dispersed granule cells in the molecular layer (m). G: In the granule cell layer (g) of a TLE patient with MCD, most of the granule cells lack, and only a few of them express CB. H: Reduction of CB immunoreactivity in the granule cell layer (g) in a patient with TUE. Scale bars: 1 mm in A–D and 100 μm in E–H.

($F(1,15) = 0.04$), digit span forward ($F(1,15) = 0.36$), Corsi span forward ($F(1,15) = 2.96$), Boston Naming test ($F(1,15) = 0.14$), and verbal fluency with F letter ($F(1,15) = 47$), A letter ($F(1,15) = 0.015$), S letter ($F(1,15) = 0.44$), or naming of animals ($F(1,15) = 0.13$), analyzed with one-way ANOVA. In addition, no significant regression could be demonstrated when rate of CB expression of patients with MCD and TUE was correlated to visual memory scores.

Using one-way ANOVA, no significant differences were seen between laterality of the focus in short-term retention ($F(1,15) = 1.9$, n.s.), long-term retention ($F(1,15) = 1.8$, n.s.), and retroactive

Table 3
Averaged neuropsychological data of the TLE patients used in this study.

Tests	Mean	SD
IQ	98.67	14.74
VQ	96.33	15
PQ	103.22	14.95
Copy of ROCF	30.64	5.64
Recall of ROCF	13.76	7.12
Trail Making A (second)	51.35	18.09
Trail Making B (second)	151.81	99.87
Digit Span	4.76	1.3
Corsi Block	3.88	1.65
AVLT I	5.12	1.4
AVLT II	7.47	1.66
AVLT III	8.12	2.26
AVLT IV	9.47	2.83
AVLT V	9.59	2.42
AVLT VI	3.82	1.07
AVLT VII	7	3.16
AVLT VIII	7.06	3.56
Boston Naming	53.65	4.04
F letter	11.06	5.3
A letter	10.12	5.09
S letter	10.53	5.34
Animals	16.53	5.56

Abbreviations: AVLT, auditory verbal learning test; VQ, verbal score of Wechsler Intelligence Scale; PQ, performance score of Wechsler Intelligence Scale; TLE, temporal lobe epilepsy; SD, standard deviation: ROCF, Rey–Osterrieth Complex Figure.

interference ($F(1,15) = 1.98$, n.s.) of AVLT. Similarly, no significant differences were found between laterality of the focus in the visual memory part of the Complex Figure test ($F(1,15) = 0.04$) and the visual-construction part of the Complex Figure test ($F(1,15) = 0.68$), Trail Making A version ($F(1,15) = 1.27$), B version ($F(1,15) = 0.72$), digit span forward ($F(1,15) = 0.024$), Corsi forward ($F(1,15) = 1.82$), verbal fluency with F letter ($F(1,15) = 0.003$), A letter ($F(1,15) = 0.42$), S letter ($F(1,15) = 0.74$), and naming of animals ($F(1,15) = 0.21$), analyzed with one-way ANOVA.

4. Discussion

The main finding of the present study is that reduction of CB immunoreactivity in the granule cells of the dentate gyrus correlates with impairment of verbal memory in patients with TLE. Significant positive association was found between CB expression in the dentate granule cells and recall phases of AVLT. Higher numbers of CB-immunoreactive granule cells were associated with better performance of immediate and delayed recall of learned verbal materials as well as with lower levels of retroactive interference.

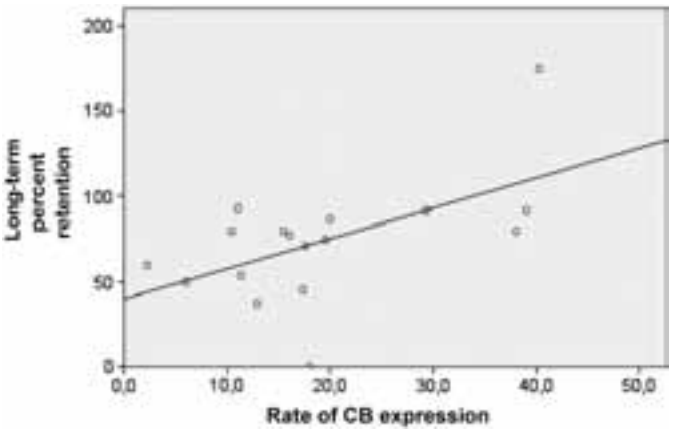

Fig. 4. Linear regression between CB expression in the granule cells of the hippocampal dentate gyrus and scores of long-term retention of AVLT ($F(1,14) = 6.03$; $p < 0.05$) of all TLE patients including patients with HS.

The role of the medial temporal lobe in verbal learning and memory is well established. Analysis of N400-evoked potentials in the left temporal lobe predicts performance of recall of learned materials in TLE patients with AVLT [63]. An event-related potential study showed the involvement of hippocampal formation and amygdala during verbal learning and memory, and amnesic patients with hippocampal lesion present greater impairment in declarative than in procedural memory [64,65]. In the literature, left HS was correlated with deficiency of verbal, right HS with impairment of visuo-spatial memory, and the severity of memory deficits was related to the degree of HS detected with MRI or ^{1}H MRS [41–43]. In addition, the degree of pyramidal cell loss in Ammon's horn and loss of hilar neurons and granule cells of the dentate gyrus correlated to memory impairments [44,45,47–49]. In the present study, we show that not only neuronal loss but also a more subtle, morphological and neurochemical alteration, namely decreased CB expression in the granule cells of the dentate gyrus, is associated with a deficit in memory functions.

The loss of CB in the granule cells is an early and common morphological sign of TLE in humans [24]. Recently, we have shown that the degree of loss of CB in the granule cells is correlated with the age of onset and the duration of TLE [19]. This observation along with that of animal models of epilepsy suggest that decrease of CB mRNA and protein expression in the granule cells is a consequence

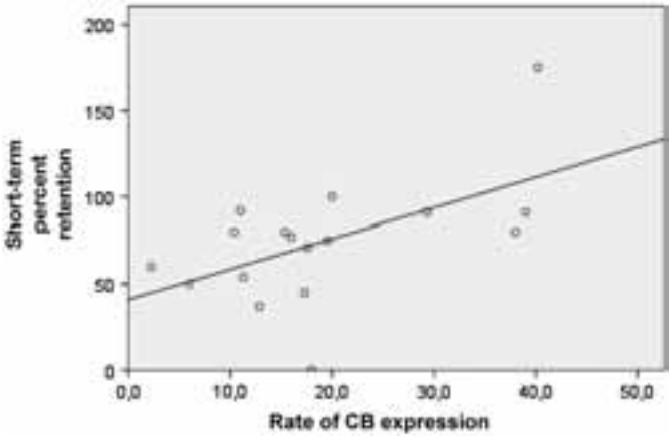

Fig. 3. Linear regression between CB expression in the granule cells of the hippocampal dentate gyrus and short-term retention scores of AVLT ($F(1,14) = 5.98$; $p < 0.05$) of all TLE patients including patients with HS.

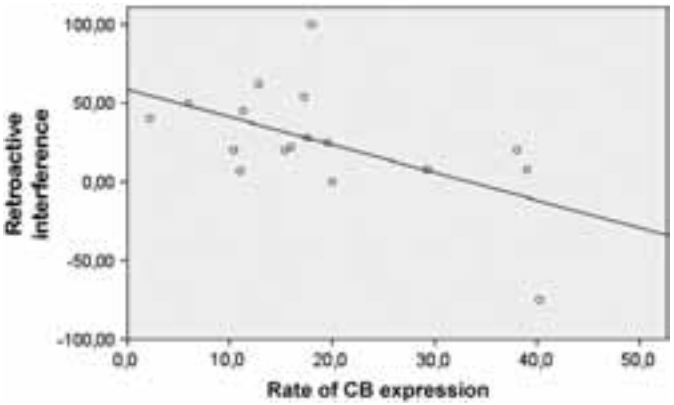

Fig. 5. Linear regression between CB expression in the granule cells of the hippocampal dentate gyrus and scores of retroactive interference of AVLT ($F(1,14) = 6.18$; $p < 0.05$) of all TLE patients including patients with HS.

Table 4
Mean and SD of the scores of auditory verbal learning tests of patients with higher rate (high-rate CB group) and lower rate (low-rate CB group) of CB-immunoreactivity in the granule cells than the average rate of CB expression.

	Mean ± SD of low-rate CB group	Mean ± SD of high-rate CB group
Short-term percent retention of AVLT	58.72 ± 25.89	102 ± 36.84
Long-term percent retention of AVLT	59 ± 26	100.05 ± 37.3
Retroactive interference of AVLT	40.94 ± 26	−2.22 ± 36.76

Abbreviations: AVLT, auditory verbal learning test; CB, calbindin; SD, standard deviation.

of neuronal hyperactivity [66]. In fact, in animal models of TLE, the decreased CB expression can be detected only ipsilaterally but not contralaterally [67]. Autopsy following TLE revealed that loss of CB in the granule cells was evident in the dentate gyrus only on the side of the focus [68].

Our results seem to contradict recent results of Martinian et al. [68] who could not detect a significant correlation between CB expression and memory function of patients with HS [68]. In HS, however, memory impairment is also influenced by neuronal loss, not only by the decreased CB expression of the granule cells. In contrast, in TLE related to MCD and tumor, neuronal loss is negligible [18,19]; therefore, it does not contribute to the loss of memory function. Our analyses, done separately on HS patients with severe neuronal loss and on patients with MCD and tumor, showed a striking difference between the two groups. While no significant correlation was found between loss of CB and memory impairment in HS, regression was significant when MCD and TUE patients, both without substantial neuronal loss, were examined. This confirms previous results showing the strong influence of neuronal loss on memory performance of HS patients [44,45,47–49] and indicates that the loss of CB in granule cells may only potentiate the impairment caused primarily by the cell loss. However, the correlation between the CB expression and the verbal memory impairment in the group of patients without substantial neuronal loss highlights the role of CB in cognitive processes such as the hippocampal learning and memory.

The role of CB in cognitive functions was established in several studies. Calbindin with its calcium buffer capacity modifies the shape and time course of intracellular free-calcium spikes [69]. Transgenic mice with reduced CB expression displayed impairment in LTP and in

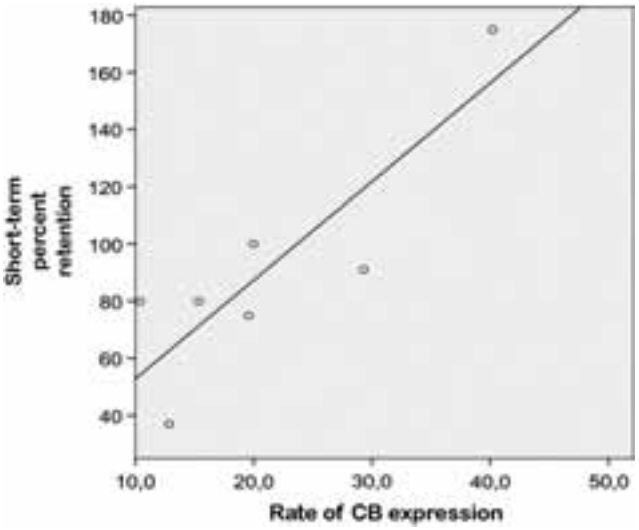

Fig. 6. Linear regression between CB expression in the granule cells of the hippocampal dentate gyrus and short-term retention scores of AVLT ($F(1,5) = 13.76$, $p < 0.05$) of the patients with TLE caused by MCD and tumor (group II).

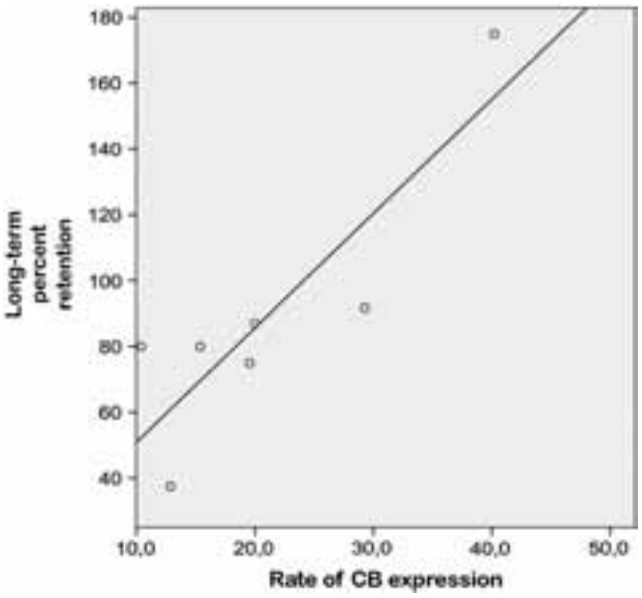

Fig. 7. Linear regression between CB expression in the granule cells of the hippocampal dentate gyrus and long-term retention scores of AVLT ($F(1,5) = 15.28$, $p < 0.05$) of the patients with TLE caused by MCD and tumor (group II).

a spatial learning test, and the memory deficit was more severe in homozygote than in heterozygote animals indicating a dependence on the amount of CB expressed [29,30]. The relation between reduction in CB expression by granule cells and memory impairment was further established in transgenic mouse models of Alzheimer's disease and fragile X syndrome. In these transgenic mice, reduced CB expression in granule cells of the dentate gyrus was linked to cognitive deficits [70–72]. In human Alzheimer's disease, severe reduction of CB immunoreactivity in dentate granule cells was observed, and the degree of reduction was age-dependent [70]. Interestingly, we have found that the reduction of CB expression in granule cells of the dentate gyrus was associated with verbal but not with visual memory deficits despite the larger numbers of individuals with a focus in the right hippocampal formation. The lack of correlation between the reduced CB immunoreactivity and the visual memory impairment can be explained by the fact that visual tasks are not as tightly hippocampus dependent as the verbal tests. Among the tests used for the visuo-spatial learning and memory, the ROCF test is considered to measure hippocampal function, while the other tasks examine attention, executive function and working memory. Despite the fact that hippocampal formation is involved in spatial working memory (e.g. in Corsi Block-Tapping test), no reliable working memory impairment could be detected in HS patients with either a left- or right-sided focus [40,73]. Studies using the ROCF test to evaluate memory deficits in epilepsy patients concluded that the ROCF test lacks the sensitivity to measure right temporal lobe function due to the possibility for verbalization of many of its components [74,75]. Furthermore, the ROCF test incorporates a wide range of cognitive abilities, such as visual perception and constructional praxis, in addition to visual memory [76]. Another explanation for the lack of association between loss of CB expression in granule cells and the visual memory deficit can be that visual tasks are less sensitive than verbal tasks. Generally, verbal memory deficits of patients with left TLE are more consistent and robust than non-verbal memory deficits in patients with right TLE [77–79].

Although the right hippocampal formation is typically associated with visuo-spatial learning and the left one is dominantly connected with declarative and verbal memory processes, indications of overlap in their functions were suggested by several studies. Structural MRI study of the hippocampal formation of London taxi drivers revealed greater gray matter volume of mid-posterior hippocampus of both

hemispheres and less gray matter in the head of both the right and the left hippocampal formations compared to controls [80]. In contrast, neuropsychological analysis indicated that taxi drivers performed better in visual memory tests than the controls; however, their verbal memory functions were not better. Deficits in topographical memory following left medial temporal lobe damage were also reported; and the left as well as the right hippocampus has been observed to be activated in some neuroimaging studies of navigation [81–86]. In addition, when figural and spatial memory was examined with the ROCF test in TLE patients, no relation could be found between memory impairment and right hippocampal volume reduction, but a strong association was found with left temporal lobe function [84,85]. In another study, the involvement of both the right and the left hippocampi was found in retrieval of autobiographical memories [86]. Similarly, impairment of retrieval of remote autobiographical memory was observed in patients with TLE with either left- or right-sided pathology [87]. In addition, left TLE patients showed selective verbal recall impairment, whereas right TLE patients had equivalent difficulty with both verbal and non-verbal recall [87]. This is in harmony with our results showing that TLE patients with either a left- or a right-sided focus exhibit deficits of verbal memory. Scores of both left and right TLE patients on short- and long-term retention and retroactive interference of AVLT were correlated with CB loss in dentate granule cells of both the left and right hippocampal formations, respectively.

Regarding the role of CB in memory, we suggest that reduction of CB expression in the granule cells might contribute to memory deficit. The granule cells of the dentate gyrus receive excitatory afferents from entorhinal cortex and form the first synapse of the trisynaptic circuitry, the structural basis of information processing allowing synaptic consolidation during learning. Therefore, alteration of their function due to decreased CB expression may be a key factor in learning and memory impairment in TLE without substantial neuronal loss, such as TLE due to MCD and TUE.

Acknowledgments

The authors wish to thank Dr. Lynn Nadel (Department of Psychology and Cognitive Science, University of Arizona, Tucson, AZ) and Dr. László Seress (Central Electron Microscopic Laboratory, University of Pécs) for critically reading and commenting on the manuscript. The excellent technical assistance of Mrs. Emese Papp in immunohistochemistry is also gratefully acknowledged. This work was supported by the Hungarian Health Research Council (grant # ETT 272/2009 to J. Janszky), Norway/EEA Financial Mechanism Grant: “Save what can be saved” (2009–2011 to J. Janszky and T. Dóczi), Developing Competitiveness of Universities in the South Transdanubian Region (SROP-4.2.1.B-10/2/KONV-2010-0002) (K. Karadi, J. Janszky, T. Dóczi), and by the Hungarian Scientific Research Fund (grant # OTKA F 68720 to J. Janszky and PD 73384 to H. Ábrahám) as well as by the Janos Bolyai Research Scholarship of the Hungarian Academy of Sciences to H. Ábrahám and J. Janszky.

References

[1] Lehericy S, Semah F, Hasboun D, et al. Temporal lobe epilepsy with varying severity: MRI study of 222 patients. Neuroradiology 1997;39:788–96.
[2] Howe KL, Dimitri D, Heyn C, Kiehl TR, Mikulis D, Valiante T. Histologically confirmed hippocampal structural features revealed by 3 T MR imaging: potential to increase diagnostic specificity of mesial temporal sclerosis. Am J Neuroradiol 2010;31:1682–9.
[3] Tassi L, Meroni A, Deleo F, et al. Temporal lobe epilepsy: neuropathological and clinical correlations in 243 surgically treated patients. Epileptic Disord 2009;11:281–92.
[4] Sommer W. Die Erkrankung des Ammonshorns als aetiologisches Moment der Epilepsie. Arch Psychiatr Nervenkr 1880;308:631–75.
[5] Dam AM. Epilepsy and neuron loss in the hippocampus. Epilepsia 1980;21:617–29.
[6] Babb TL, Brown WJ, Pretorius J, Davenport C, Lieb JP, Crandall PH. Temporal lobe volumetric cell densities in temporal lobe epilepsy. Epilepsia 1984;25:729–40.
[7] Blümcke I, Suter B, Behle K, et al. Loss of hilar mossy cells in Ammon's horn sclerosis. Epilepsia Suppl 2000;6:S174–80.
[8] Cronin J, Dudek FE. Chronic seizures and collateral sprouting of dentate mossy fibers after kainic acid treatment in rats. Brain Res 1988;474:181–4.
[9] Represa A, Le Gall La Salle G, Ben-Ari Y. Hippocampal plasticity in the kindling model of epilepsy in rats. Neurosci Lett 1989;99:345–50.
[10] de Lanerolle NC, Kim JH, Robbins RJ, Spencer DD. Hippocampal interneuron loss and plasticity in human temporal lobe epilepsy. Brain Res 1989;495:387–95.
[11] Sutula T, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. Ann Neurol 1989;26:321–30.
[12] Davenport CJ, Brown WJ, Babb TL. Sprouting of GABAergic and mossy fiber axons in dentate gyrus following intrahippocampal kainate in the rat. Exp Neurol 1990;109:180–90.
[13] Houser CR, Miyashiro JE, Swartz BE, Walsh GO, Rich JR, Delgado-Escueta AV. Altered patterns of dynorphin immunoreactivity suggest mossy fiber reorganization in human hippocampal epilepsy. J Neurosci 1990;10:267–82.
[14] Maglóczky Z, Wittner L, Borhegyi Z, et al. Changes in the distribution and connectivity of interneurons in the epileptic human dentate gyrus. Neuroscience 2000;96:7–25.
[15] Wittner L, Eross L, Szabó Z, et al. Synaptic reorganization of calbindin-positive neurons in the human hippocampal CA1 region in temporal lobe epilepsy. Neuroscience 2002;115:961–78.
[16] Kim JH, Guimaraes PO, Shen MY, Masukawa LM, Spencer DD. Hippocampal neuronal density in temporal lobe epilepsy with and without gliomas. Acta Neuropathol 1990;80:41–5.
[17] Blümcke I, Beck H, Lie AA, Wiestler OD. Molecular pathology of human mesial temporal lobe epilepsy. Epilepsy Res 1999;36:205–23.
[18] de Lanerolle NC, Kim JH, Williamson A, et al. A retrospective analysis of hippocampal pathology in human temporal lobe epilepsy: evidence for distinctive patient subcategories. Epilepsia 2003;44:677–87.
[19] Abrahám H, Richter Z, Gyimesi C, et al. Degree and pattern of calbindin immunoreactivity in granule cells of the dentate gyrus differ in mesial temporal sclerosis, cortical malformation- and tumor-related epilepsies. Brain Res 2011;1399:66–78.
[20] Awad IA, Rosenfeld J, Ahl J, Hahn JF, Lüders H. Intractable epilepsy and structural lesions of the brain: mapping, resection strategies, and seizure outcome. Epilepsia 1991;32:179–86.
[21] Berger MS, Ghatan S, Haglund MM, Dobbins J, Ojemann GA. Low-grade gliomas associated with intractable epilepsy: seizure outcome utilizing electrocorticography during tumor resection. J Neurosurg 1993;79:62–9.
[22] Cascino GD, Jack Jr CR, Parisi JE, et al. Operative strategy in patients with MRI-identified dual pathology and temporal lobe epilepsy. Epilepsy Res 1993;14:175–82.
[23] Mathern GW, Babb TL, Pretorius JK, Melendez M, Lévesque MF. The pathophysiologic relationships between lesion pathology, intracranial ictal EEG onsets, and hippocampal neuron losses in temporal lobe epilepsy. Epilepsy Res 1995;21:133–47.
[24] Maglóczky ZS, Halász P, Vajda J, Czirják S, Freund TF. Loss of calbindin-D28K immunoreactivity from dentate granule cells in human temporal lobe epilepsy. Neuroscience 1997;76:377–85.
[25] Nagerl UV, Mody I. Calcium-dependent inactivation of high-threshold calcium currents in human dentate gyrus granule cells. J Physiol 1998;509:39–45.
[26] Nägerl UV, Mody I, Jeub M, Lie AA, Elger CE, Beck H. Surviving granule cells of the sclerotic human hippocampus have reduced Ca(2+) influx because of a loss of calbindin-D(28k) in temporal lobe epilepsy. J Neurosci 2000;20:1831–6.
[27] Bouilleret V, Schwaller B, Schurmans S, Celio MR, Fritschy JM. Neurodegenerative and morphogenic changes in a mouse model of temporal lobe epilepsy do not depend on the expression of the calcium-binding proteins parvalbumin, calbindin, or calretinin. Neuroscience 2000;97:47–58.
[28] Gary DS, Sooy K, Chan SL, Christakos S, Mattson MP. Concentration- and cell type-specific effects of calbindin D28k on vulnerability of hippocampal neurons to seizure-induced injury. Brain Res Mol Brain Res 2000;75:89–95.
[29] Molinari S, Battini R, Ferrari S, et al. Deficits in memory and hippocampal long-term potentiation in mice with reduced calbindin D28K expression. Proc Natl Acad Sci U S A 1996;93:8028–33.
[30] Jouvenveau A, Potier B, Poindessous-Jazat F, et al. Decrease in calbindin content significantly alters LTP but not NMDA receptor and calciun channel properties. Neuropharmacology 2002;42:444–58.
[31] Nadel L, Willner J. Some implications of postnatal maturation of hippocampus. In: Chan-Palay V, Köhler C, Liss AR, editors. The hippocampus. New York: New Vistas; 1989. p. 17–31.
[32] Ábrahám H, Orsi G, Seress L. Ontogeny of cocaine- and amphetamine-regulated transcript (CART) peptide and calbindin immunoreactivity in granule cells of the dentate gyrus in the rat. Int J Dev Neurosci 2007;25:265–74.
[33] Overman WH, Pate BJ, Moore K, Peuster A. Ontogeny of place learning in children as measured in the radial arm maze, Morris search task, and open field task. Behav Neurosci 1996;110:1205–28.
[34] Ábrahám H, Veszprémi B, Kravják A, Kovács K, Gömöri E, Seress L. Ontogeny of calbindin immunoreactivity in the human hippocampal formation with a special emphasis on granule cells of the dentate gyrus. Int J Dev Neurosci 2009;27:115–27.
[35] Collier TJ, Routtenberg A. Selective impairment of declarative memory following stimulation of dentate gyrus granule cells: a naloxone-sensitive effect. Brain Res 1984;310:384–7.
[36] Cave CB, Squire LR. Equivalent impairment of spatial and nonspatial memory following damage to the human hippocampus. Hippocampus 1991;1:329–40.
[37] Mameniskiene R, Jatuzis D, Kaubrys G, Budry V. The decay of memory between delayed and long-term recall in patients with temporal lobe epilepsy. Epilepsy Behav 2006;8:278–88.

[38] Marques CM, Caboclo LOSF, da Silva TI, et al. Cognitive decline in temporal epilepsy due to unilateral hippocampal sclerosis. Epilepsy Behav 2007;10:477–85.
[39] Herfurth K, Kasper B, Schwarz M, Stefan H, Pauli E. Autobiographical memory in temporal lobe epilepsy: role of hippocampal and temporal lateral structures. Epilepsy Behav 2010;19:365–71.
[40] Tudesco Ide S, Vaz LJ, Mantoan MA, et al. Assessment of working memory in patients with mesial temporal lobe epilepsy associated with unilateral hippocampal sclerosis. Epilepsy Behav 2010;18:223–8.
[41] Helmstaedter C, Wietzke J, Lutz MT. Unique and shared validity of the "Wechsler logical memory test", the "California verbal learning test", and the "verbal learning and memory test" in patients with epilepsy. Epilepsy Res 2009;87:203–12.
[42] Alessio A, Damasceno BP, Camargo CHP, Kobayashi E, Guerreiro CAM, Cendes F. Differences in memory performance and other clinical characteristics in patients with mesial temporal lobe epilepsy with and without hippocampal atrophy. Epilepsy Behav 2004;5:22–7.
[43] Hanoglu L, Ozkara C, Keskinkiliç C, et al. Correlation between ^{1}H MRS and memory before and after surgery in mesial temporal lobe epilepsy with hippocampal sclerosis. Epilepsia 2004;45:632–40.
[44] Sass KJ, Spencer DD, Kim JH, Westerveld M, Novelly RA, Lencz T. Verbal memory impairment correlates with hippocampal pyramidal cell density. Neurology 1990;40:1694–7.
[45] Sass KJ, Sass A, Westerveld M, et al. Specificity in the correlation of verbal memory and hippocampal neuron loss: dissociation of memory, language, and verbal intellectual ability. J Clin Exp Neuropsychol 1992;14:662–72.
[46] Sass KJ, Westerveld M, Buchanan CP, Spencer SS, Kim JH, Spencer DD. Degree of hippocampal neuron loss determines severity of verbal memory decrease after left anteromesiotemporal lobectomy. Epilepsia 1994;35:1179–86.
[47] Sass KJ, Buchanan CP, Kraemer S, Westerveld M, Kim JH, Spencer DD. Verbal memory impairment resulting from hippocampal neuron loss among epileptic patients with structural lesions. Neurology 1995;45:2154–8.
[48] Rausch R, Babb TL. Hippocampal neuron loss and memory scores before and after temporal lobe surgery for epilepsy. Arch Neurol 1993;50:812–7.
[49] Pauli E, Hildebrandt M, Romstöck J, Stefan H, Blümcke I. Deficient memory acquisition in temporal lobe epilepsy is predicted by hippocampal granule cell loss. Neurology 2006;67:1383–9.
[50] Coras R, Siebzehnrubl FA, Pauli E, et al. Low proliferation and differentiation capacities of adult hippocampal stem cells correlate with memory dysfunction in humans. Brain 2010;133:3359–72.
[51] Lezak MD, Howieson DB, Loring DW. Neuropsychological assessment. Oxford: Oxford University Press; 2004.
[52] Hodges JR. Cognitive assessment for clinicians. Oxford: Oxford Uviversity Press; 1996.
[53] Mitrushina MN, Boone KB, D'Elia LF. Handbook of normative data for neuropsychological assessment. Oxford: Oxford University Press; 1999.
[54] Lilliwhite LM, Saling MM, Briellman RS, Weintrob DL, Pell GS, Jackson GD. Differential contributions of the hippocampus and rhinal cortices to verbal memory in epilepsy. Epilepsy Behav 2007;10:553–9.
[55] Abrahám H, Tornóczky T, Kosztolányi G, Seress L. Cell formation in the cortical layers of the developing human cerebellum. Int J Dev Neurosci 2001;19:53–62.
[56] Seress L, Abrahám H, Tornóczky T, Kosztolányi G. Cell formation in the human hippocampal formation from mid-gestation to the late postnatal period. Neuroscience 2001;105:831–43.
[57] Sloviter RS, Kudrimoti HS, Laxer KD, et al. "Tectonic" hippocampal malformations in patients with temporal lobe epilepsy. Epilepsy Res 2004;59:123–53.
[58] Wyler AR, Dohan Jr FC, Schweitzer JB, Berry III AD. A grading system for mesial temporal pathology (hippocampal sclerosis) from anterior temporal lobectomy. J Epilepsy 1992;5:220–5.
[59] Thom M, Liagkouras I, Elliot KJ, et al. Reliability of patterns of hippocampal sclerosis as predictors of postsurgical outcome. Epilepsia 2010;51:1801–8.
[60] Blümcke I, Pauli E, Clusmann H, et al. A new clinico-pathological classification system for mesial temporal sclerosis. Acta Neuropathol 2007;113:235–44.
[61] Blümcke I, Kistner I, Clusmann H, et al. Towards a clinico-pathological classification of granule cell dispersion in human mesial temporal lobe epilepsies. Acta Neuropathol 2009;117:535–44.
[62] Blümcke I, Thom M, Aronica E, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. Epilepsia 2011;52:158–74.
[63] Elger CE, Grunwald T, Lehnertz K, et al. Human temporal lobe potentials in verbal learning and memory processes. Neuropsychologia 1997;35:657–67.
[64] Babiloni C, Vecchio F, Mirabella G, et al. Activity of hippocampal, amygdala, and neocortex during the Rey auditory verbal learning test: an event-related potential study in epileptic patients. Clin Neurophysiol 2010;121:1351–7.
[65] Hopkins RO, Waldram K, Kesner RP. Sequences assessed by declarative and procedural tests of memory in amnesic patients with hippocampal damage. Neuropsychologia 2004;42:1877–86.
[66] Carter DS, Harrison AJ, Falenski KW, Blair RE, DeLorenzo RJ. Long-term decrease in calbindin-D28K expression in the hippocampus of epileptic rats following pilocarpine-induced status epilepticus. Epilepsy Res 2008;79:213–23.
[67] Magloczky Z, Freund TF. Selective neuronal death in the contralateral hippocampus following unilateral kainate injections into the CA3 subfield. Neuroscience 1993;56:317–35.
[68] Martinian L, Catarino CB, Thompson P, Sisodiya SM, Thom M. Calbindin D28K expression in relation to granule cell dispersion, mossy fibre sprouting and memory impairment in hippocampal sclerosis: a surgical and post mortem series. Epilepsy Res 2012;98:14–24.
[69] Lledo PM, Somasundaram B, Morton AJ, Emson PC, Mason WT. Stable transfection of calbindin-D28k into the GH3 cell line alters calcium currents and intracellular calcium homeostasis. Neuron 1992;9:943–54.
[70] Palop JJ, Jones B, Kekonius L, et al. Neuronal depletion of calcium-dependent proteins in the dentate gyrus is tightly linked to Alzheimer's disease-related cognitive deficits. Proc Natl Acad Sci U S A 2003;100:9572–7.
[71] Odero GL, Oikawa K, Glazner KA, et al. Evidence for the involvement of calbindin D28k in the presenilin 1 model of Alzheimer's disease. Neuroscience 2010;169:532–43.
[72] Real MA, Simón MP, Heredia R, de Diego Y, Guirado S. Phenotypic changes in calbindin D28K immunoreactivity in the hippocampus of Fmr1 knockout mice. J Comp Neurol 2011;519:2622–36.
[73] Toepper M, Markowitsch HJ, Gebhardt H, et al. Hippocampal involvement in working memory encoding of changing locations: an fMRI study. Brain Res 2010;1354:91–9.
[74] Kneebone AC, Lee GP, Wade LT, Loring DW. Rey Complex Figure: figural and spatial memory before and after temporal lobectomy for intractable epilepsy. J Int Neuropsychol Soc 2007;13:664–71.
[75] McConley R, Martin R, Palmer CA, Kuzniecky R, Knowlton R, Faught E. Rey Osterrieth complex figure test spatial and figural scoring: relations to seizure focus and hippocampal pathology in patients with temporal lobe epilepsy. Epilepsy Behav 2008;13:174–7.
[76] Waber DP, Holmes JM. Assessing children's memory productions of the Rey–Osterrieth Complex Figure. J Clin Exp Neuropsychol 1986;8:563–80.
[77] Barr WB. Examining the right temporal lobe's role in nonverbal memory. Brain Cogn 1997;35:26–41.
[78] Baxendale SA, Thompson PJ, Van Paesschen W. A test of spatial memory and its clinical utility in the pre-surgical investigation of temporal lobe epilepsy patients. Neuropsychologia 1998;36:591–602.
[79] Raspall T, Donate M, Boget T, et al. Neuropsychological tests with lateralizing value in patients with temporal lobe epilepsy: reconsidering material-specific theory. Seizure 2005;14:569–76.
[80] Maguire EA, Woollett K, Spiers HJ. London taxi drivers and bus drivers: a structural MRI and neuropsychological analysis. Hippocampus 2006;16:1091–101.
[81] Maguire EA, Burke T, Phillips J, Staunton H. Topographical disorientation following unilateral temporal lobe lesions in humans. Neuropsychologia 1996;34:993–1001.
[82] Maguire EA, Burgess N, Donnett JG, Frackowiak RS, Frith CD, O'Keefe J. Knowing where and getting there: a human navigation network. Science 1998;280:921–4.
[83] Ghaem O, Mellet E, Crivello F, et al. Mental navigation along memorized routes activates the hippocampus, precuneus, and insula. Neuroreport 1997;8:739–44.
[84] Grön G, Wunderlich AP, Spitzer M, Tomczak R, Riepe MW. Brain activation during human navigation: gender-different neural networks as substrate of performance. Nat Neurosci 2000;3:404–8.
[85] Spiers HJ, Maguire EA. Thoughts, behaviour, and brain dynamics during navigation in the real world. Neuroimage 2006;31:1826–40.
[86] Maguire EA, Frith CD. Lateral asymmetry in the hippocampal response to the remoteness of autobiographical memories. J Neurosci 2003;23:5302–7.
[87] Viskontas IV, McAndrews MP, Moscovitch M. Remote episodic memory deficits in patients with unilateral temporal lobe epilepsy and excisions. J Neurosci 2000;20:5853–7.