

FULL-LENGTH ORIGINAL RESEARCH

Increased leak conductance in dentate gyrus granule cells of temporal lobe epilepsy patients with Ammon's horn sclerosis

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SUMMARY

Purpose: Temporal lobe epilepsy (TLE) is often associated with Ammon's horn sclerosis (AHS) characterized by hippocampal cell death and dentate gyrus granule cell dispersion (GCD). Granule cells survive AHS and have been proposed to be hyperexcitable in TLE. Here we studied whether the passive excitability of granule cells correlates with the severity of AHS.

Methods: We analyzed the passive membrane properties of identified granule cells using patch-clamp recordings in acute tissue slices obtained from TLE surgery. Independent Wyler grading and GCD measurements were used to assess the severity of AHS.

Results: The input resistances and membrane time constants of granule cells were reduced in high-grade versus low-grade AHS samples and negatively correlated with the degree of GCD. Granule cells possessed large Ba^{2+} -sensitive, inwardly rectifying K^+ conductances.

Discussion: The increased leak conductance, likely mediated by K^+ channels, does not argue for an increased excitability of granule cells but rather points to a neuroprotective mechanism in the sclerotic focus in TLE.

KEY WORDS: Mesial temporal lobe epilepsy, Brain slice preparations, Neuroprotective strategies, Ion channel electrophysiology, Potassium channels.

Mesial temporal lobe epilepsy (TLE), the most prevalent form of focal epilepsies, is often intractable, but surgical resection of the hippocampus and adjacent medial temporal structures leads to seizure cessation in most cases (Zentner et al., 1995). Resected hippocampi often show Ammon's horn sclerosis (AHS) characterized by selective cell death, gliosis, and network disorganization (Margerison & Corsellis, 1966; Blumcke et al., 2002). Closely linked with the progression of AHS is the granule cell dispersion (GCD) in the dentate gyrus (Houser,

1990; Thom et al., 2002). Granule cells survive AHS and have frequently been implicated in the generation of seizure activity (Tauk & Nadler, 1985; Sloviter, 1991; Heinemann et al., 1992; Remy et al., 2003). However, whether granule cells become intrinsically hyperexcitable and actively contribute to TLE seizures is not securely established (Dudek et al., 1994; Sloviter, 1994; Jefferys, 1999; Liu et al., 2000; Harvey & Sloviter, 2005; Naegele, 2007). It is also an open question why granule cells survive AHS and other excitotoxic conditions better than neighboring neuronal populations (Olney et al., 1979; Ben-Ari, 1985; Magloczky et al., 1997; Bouilleret et al., 2000; Nagerl et al., 2000).

Upregulation of leak K^+ conductances is a potentially neuroprotective and anticonvulsive mechanism that reduces cellular excitability (Leblond & Krnjevic, 1989; Brickley et al., 2001; Wickenden, 2002), but it is unknown whether such changes occur in granule cells during TLE. In particular, no data exist on the relation of

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the electrophysiologic properties of granule cells and progressive GCD. To address this question we studied human granule cells using patch-clamp recordings in acute hippocampal slices obtained from TLE surgery. We found that with increasing GCD, granule cells displayed an increasing leak conductance, likely carried by K⁺ ions. These results are relevant to the understanding of seizure mechanisms in TLE, because they suggest that the passive excitability of granule cells in the sclerotic focus is decreased rather than increased.

METHODS

Patient data

All procedures on human tissue were approved by the ethics committee of the University of Freiburg. In all cases, surgical removal of the hippocampus was indicated clinically and written informed consent about electrophysiologic and histologic studies was obtained. Clinical char-

acteristics of patients are summarized in Table 1. One cell of a 4-year-old patient was included, which did not induce a bias toward early developmental stages. Patient data are based on information available in discharge letters, and consequently may be incomplete or based on estimates. For some values of seizure frequency and duration a mean was calculated from the available range.

Brain slice preparation

Immediately after resection, human hippocampi were immersed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 87 NaCl, 25 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 0.5 CaCl₂, 7 MgCl₂, 75 sucrose, and 10 glucose (equilibrated with 95% O₂/5% CO₂). For neuropathologic analysis, slices of 1–3 mm thickness were cut and subsequently one of these was further cut into slices of 400 μm with a vibratome (VT1000S or VT1200S, Leica, Bensheim, Germany) for electrophysiologic experiments. Slices were incubated in sucrose ACSF for 30 min at

Table 1. Patient data

Age OP	Sex	Age onset	Seizure		H	AED treatment	Additional information/syndromes	Wy
			Type	Frequency				
49	M	2.6	SP/CP/SG	10	R	n.i.	Organic brain syndrome, newborn spasm, cyanosis	3
56	M	45.7	SP/CP/ISG	4	R	LEV, VPA	Alcohol misuse	3
28	F	0.4	CP/SG	6	L	ZNS, OXC	FD of cortex and amygdala	2.5
14	M	5.8	SP/CP/ISG	16	L	LTG	FCD type 2a	3
17	M	4.8	SP/CP	2	R	LTG, ZNS, PB	FCD 1b. Encephalitis, connatal hydrocephalus	3
60	M	17.7	SP/CP/SG	4	L	LEV, LTG	Hypocalcemic tetany	3
49	F	7.8	SP/CP/SG	12	L	LEV, CLB, SER	Febrile seizure in childhood	3
51	F	6.3	SP/CP/SG	8	L	CLB, LEV	FCD 1b	2.5
28	F	6.4	SP/CP	30	R	CLB, LEV, CLB	FCD 2a, febrile seizures in childhood	3
4	M	0.7	SP/CP	2.5	R	OXC, STM	FCD 2a, intrauterine cerebral hemorrhage	2
43	F	14.0	SP/CP/SG	15	R	LTG, LEV	FCD 1a	3
55	F	18.8	SP/CP/SG	7	L	CLB, STM, CLO	FCD 2a, meningitis in childhood	2
32	M	12.8	CP	40	R	LEV, LTG	FCD 1b	3.5
25	F	0.8	SP/CP/SG	3	L	n.i.	Encephalitis in childhood	3
27	M	21.7	SP/CP	1	L	LEV	FCD 2a	2
19	F	10.4	SP/CP	45	R	OXC, TPM, VPA	Ganglioglioma	2
51	F	44.6	SP/CP/SG	2	R	LEV, ZNS	Falx meningioma, 2003 TBE	3
26	F	1.3	SP/CP/SG	10	L	OXC, LTG	Peripartur hypoxia, preterm birth, febrile seizures in childhood	4
56	M	1.0	SP/CP/SG	5	R	PHT	FCD 2a, eclampsia of mother, first seizure after smallpox vaccination	4
35	M	0.1	SP/CP/SG	6	R	OXC, LTG	Pneumonia in fifth postnatal week with artificial respiration, meningo-encephalitis, spastic hemiparesis	4
48	M	34	SP/CP/ISG	20	L	LTG	Febrile seizures in childhood, head injury, left parietooccipital atrophy	
45	F	8.6	SP/CP/SG	4	L	LEV, LTG, CLB	Craniocerebral injury with 8 years	3

Age OP, age at resection of hippocampus; Age onset, age at epilepsy onset; CP, complex partial seizures; SP, simple partial seizures; SG, secondary generalized seizures; H, hemisphere of resected hippocampus; AED, antiepileptic drug; CBZ, carbamazepine; CLB, clobazam; LEV, levetiracetam; LTG, lamotrigine; OXC, oxcarbazepine; PB, phenobarbital; PHT, phenytoin; STM, sulthiame; TPM, topiramate; VPA, valproate; ZNS, zonisamide; n.i., no information; FD/FCD, focal cortical dysplasia; TBE, tick-borne encephalitis; Wy, Wyler grade.

$35.5 \pm 1^\circ\text{C}$ and subsequently kept at room temperature ($23 \pm 1^\circ\text{C}$) in oxygenated sucrose ACSF for >1 h until they were transferred individually for electrophysiologic experiments.

Electrophysiology

Patch-clamp methods were adopted from Schmidt-Hieber et al. (2004). For patch-clamp recordings, tissue slices were transferred to a recording chamber and continuously superfused at room temperature with glucose ACSF containing (in mM): 125 NaCl, 25 NaHCO_3 , 2.5 KCl, 1.25 NaH_2PO_4 , 2 CaCl_2 , 1 MgCl_2 , and 25 glucose (equilibrated with 95% O_2 /5% CO_2). Cells were visualized by infrared differential interference contrast video microscopy using a $63\times/1.0$ objective in an upright microscope (Axioskop2 FS, Zeiss, Oberkochen, Germany). Patch pipettes were pulled from borosilicate glass using a DMZ-universal puller (Zeitz, Martinsried, Germany). They were filled with a solution containing (in mM): 135 Kgluconate, 20 KCl, 10 HEPES, 0.1 EGTA, 2 MgCl_2 , 2 Na_2ATP and 0.2% biocytin (pH = 7.28) and had tip resistances of 5.1 ± 0.7 M Ω . To obtain optimal biocytin-labeling, the whole-cell configuration was maintained for at least 25 min and the pipette was retrieved via the outside-out configuration. Records were filtered at 10 kHz and 8 kHz using a SEC05LX amplifier (NPI, Tamm, Germany) and digitized at 20 kHz and 10 kHz (current and voltage clamp, respectively) using a ITC18 D/A converter (Instrutech, Port Washington, NY, U.S.A.) and the software PatchMaster (Heka, Lambrecht, Germany). Series resistance (7–16 M Ω) was compensated via bridge balance, and pipette capacitance was compensated via fast capacitance controls of the single electrode clamp (SEC) amplifier. The ratio of seal resistance (>1 G Ω) to input resistance (R_{in}) ranged between 2.3 and 39.3. The liquid junction potential was determined to be 10 mV (Neher, 1992) and voltages were appropriately corrected offline. Most pharmacologic experiments were conducted in the presence of tetrodotoxin (TTX) and synaptic blockers, that is, D(-)-2-amino-5-phosphonopentanoic acid (D-AP5), 1,2,3,4-tetrahydro-7-nitro-2,3-dioxoquinoxaline-6-carbonitrile disodium (CNQX), and picrotoxin (PTX). In K^+ -replacement experiments, cells were first recorded with normal intracellular solution and (via the outside-out configuration) subsequently repatched with K^+ -free intracellular solution. In these cases K^+ was replaced equimolar with tetraethylammonium (TEA) in the ACSF and the pipette solution contained (in mM): 135 TEACl, 20 CsCl, 0.1 EGTA, 2 MgCl_2 , 2 Na_2ATP and 0.2% biocytin (pH 7.28 adjusted with TEA hydroxide). CNQX, D-AP5, TEACl, and TTX were kept in H_2O stocks at -20°C and PTX was kept in dimethylsulfoxide (DMSO) stocks at -20°C . Final concentrations were diluted freshly in oxygenated

recording ACSF (DMSO 1:1,000) and subsequently kept in glass syringes of an application system (Auto-Mate Scientific, Berkeley, CA, U.S.A.) under carbogen pressurized at 1.3–1.6 bar before bath application. We obtained D-AP5, TTX, and CNQX disodium salt from Ascent Scientific (Weston-Super-Mare, United Kingdom) and KCl from Merck (Darmstadt, Germany). All other substances were purchased from Sigma–Aldrich (Taufkirchen, Germany).

Morphologic reconstructions and immunocytochemistry

For localization and identification of patched granule cells we used fluorescence labeling and immunocytochemistry. Slices were fixed overnight with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) pH 7.4. After three washing steps with PB, slices were treated for 30 min with a blocking solution containing 0.3% Triton X-100 and 10% NGS in 0.1 M PB at room temperature, followed by incubation with a rabbit polyclonal anti-Prox1 antibody (1:1000, Chemicon, Temecula, CA, U.S.A.) in 0.1% Triton and 1% NGS either for >3 h at room temperature or overnight at 4°C . After three washes, slices were incubated with Alexa Fluor-546-Streptavidin (1:500) and a secondary anti-rabbit antibody conjugated with Alexa Fluor-488 (1:200, Invitrogen, Karlsruhe, Germany), either for >3 h at room temperature or overnight at 4°C . After five washes, slices were mounted in fluorescence mounting medium (DAKO, Glostrup, Denmark) or ProLong gold antifade reagent (Invitrogen). Immunofluorescence was analyzed with an Axioplan 2 microscope equipped with Apotome technology (Zeiss) using the $10\times/0.45$ objective and extended focal images.

GCD measurements and Wyler grading

As a measurement for the degree of AHS we used the Wyler grading in which parameters such as hippocampal cell loss and astrogliosis are judged by a neuropathologist (Wyler et al., 1992). In this classification, low-grade lesions (grades 1–2) correspond to mild and moderate hippocampal damage, whereas high-grade lesions (grades 3–4) correspond to moderate (“classical AHS”) and marked (“total AHS”) hippocampal damage (Wyler et al., 1992). One patient had a presumed extrahippocampal epileptogenic lesion (ganglioglioma), a situation sometimes considered a control to AHS, but the pathology report also determined moderate hippocampal damage. GCD was quantified as width of granule cell layer measured at the position and focal level of reconstructed neurons. For two cells that could not be doubtlessly identified upon reconstruction, GCD was measured at least at three positions in the granule cell layer and averaged for each slice (Haas et al., 2002). GCD was reasonably homogenous within slices except for one case that was excluded from this analysis. Neither the pathologist nor the experimenters

performing the GCD measurements were aware of the respective electrophysiologic results.

Data analysis

Electrophysiologic records were analyzed using Igor-Pro (WaveMetrics, Portland, OR, U.S.A.) and FitMaster (Heka). Only cells with resting membrane potentials negative to -60 mV and overshooting action potentials were included in the analysis. The R_{in} was calculated from the slope of the steady-state current–voltage (I/V) relation from voltage responses of less than ± 10 mV from resting membrane potential. The membrane time constant (τ_m) was derived by the average of 4–6 single exponentials fitted to voltage responses to 450-ms current steps. The resting conductance was calculated as the reciprocal of the slope of the fitted current voltage relation (four steps of 3 mV at holding potentials of -80 or -90 mV). Statistical significance of group differences was calculated using the software PRISM 3.0 (GraphPad, San Diego, CA, U.S.A.) applying the following tests: Mann-Whitney test for two groups not normally distributed, Student's t -tests for two groups normally distributed, and paired t -tests and Wilcoxon signed rank test for paired comparisons. Significance of correlation was determined according to a table of Pearson's r -values. All mean values are \pm SEM (standard error of the mean). Numbers represent cells if not mentioned otherwise. Figures were produced using GraphPad, Adobe Illustrator, and Adobe Photoshop (Adobe, München, Germany).

RESULTS

The input resistance of granule cells correlates with the degree of granule cell dispersion

We recorded from granule cells of hippocampi resected from 22 TLE patients, whose clinical characteristics are summarized in Table 1. To ensure that recorded cells were granule cells, they were labeled with biocytin during recordings for post hoc anatomic reconstructions and immunocytochemical identification. All recovered cells were positioned within the granule cell layer and had spiny apical dendrites extending into the molecular layer (Fig. 1A). In addition, cells were co-labeled with Prox1, a granule cell marker (Fig. 1A) (Liu et al., 2000). Prox1-negative cells were excluded from further analysis. Granule cells displayed resting membrane potentials (V_m) of -74.5 ± 0.81 mV, R_{in} of 222 ± 18 M Ω , action potential thresholds of -47.0 ± 1.0 mV, and membrane time constants (τ_m) of 27.4 ± 1.6 ms ($n = 46$).

To relate the degree of AHS of hippocampal specimen to our electrophysiologic recordings, AHS classification according to Wyler was used (see Methods) (Wyler et al., 1992). We grouped our data into low-grade lesions (Wyler grade 2–2.5, Wy_2) and high-grade lesions, that is, classical and total AHS (Wyler grade 3–4, Wy_3) (Fig. 1B). The

V_m was similar in these groups (Fig. 1C) (Wy_2 , -73.2 ± 1.1 mV, $n = 14$; Wy_3 , -75.1 ± 1.1 mV, $n = 32$, $p = 0.23$). However, the input resistance (R_{in}) was clearly reduced in the Wy_3 group (Fig. 1D,E) (Wy_2 , 290 ± 41 M Ω , $n = 14$; Wy_3 , 192 ± 16 M Ω , $n = 32$, $p < 0.05$). Similarly, τ_m was reduced in the Wy_3 group (Fig. 1F) (Wy_2 , 34.6 ± 3.1 ms, $n = 14$; Wy_3 , 24.3 ± 1.6 ms, $n = 32$, $p < 0.001$).

As a second measure related to the degree of AHS (Thom et al., 2002), we measured the width of granule cell layer (GCD, see Methods) and divided the data into “weak GCD” (D_w , 54–116 μ m, Fig. 1B left panel) and “strong GCD” (D_s , width of granule cell layer 131–315 μ m, Fig. 1B right panel). Similar to the results using the Wyler grading, the V_m was similar in the GCD groups (Fig. 1G) (D_w , -72.1 ± 1.4 mV, $n = 10$; D_s , -75.2 ± 0.9 mV, $n = 36$, $p = 0.15$), but R_{in} and τ_m were significantly reduced in the D_s group (Fig. 1H,I) (D_w , R_{in} , 299 ± 53 M Ω , τ_m , 34.9 ± 4.6 ms, $n = 10$; D_s , R_{in} , 201 ± 16 M Ω , τ_m , 25.4 ± 1.5 ms, $n = 36$, respectively, $p < 0.05$).

As a third form of analysis, parameters were plotted directly against GCD. Both R_{in} and τ_m correlated with the severity of GCD (Fig. 1J) (τ_m , $r = 0.53$, R_{in} not shown, $r = 0.50$, $n = 18$ slices with 44 cells, respectively, $p < 0.05$). Interestingly, R_{in} and τ_m also correlated with the frequency of complex partial seizures per month (data not shown) (τ_m , $r = 0.48$, R_{in} , $r = 0.53$, $n = 18$ slices with 44 cells, $p < 0.05$). However, this seizure frequency information should be treated with caution because it was not directly measured (see Methods).

Granule cells with low-input resistance display large Ba^{2+} -sensitive K^+ leak conductances

To obtain more information about the mechanism underlying the decreased R_{in} of dispersed granule cells, we characterized the resting conductance (g_{rest}) defined here as the conductance measured in voltage-clamp mode close to the V_m (see Methods). When the intracellular and extracellular K^+ was replaced with TEA $^+$ (“zero K^+ ”) the cells depolarized and their g_{rest} was strongly reduced (Fig. 2A) (control, 5.6 ± 0.9 nS, zero K^+ , 2.3 ± 0.4 , $n = 4$, $p < 0.05$). These results give an estimate of the K^+ conductance controlling the V_m of human granule cells (3.3 ± 0.8 nS). The amount of resting K^+ conductance correlated with the initial R_{in} of the respective cells (Fig. 2B) ($r = 0.97$, $p < 0.05$). Consistent with flux through K^+ channels these currents reversed at -97 mV, close to the Nernst equilibrium potential of K^+ ions (Fig. 2C) (-104 mV).

The K^+ channel inhibitor Ba^{2+} (1 mM) depolarized granule cells (from -73.9 ± 1.5 mV to -52.4 ± 3.1 mV, $n = 7$, $p < 0.001$) and decreased the g_{rest} of granule cells to less than half of its initial value (Fig. 2D) (from 6.1 ± 0.6 nS to 2.6 ± 0.3 nS, $n = 7$, $p < 0.05$; Ba^{2+} -sensitive g_{rest} , 3.5 ± 1.1 nS). The amount of Ba^{2+} -sensitive g_{rest}

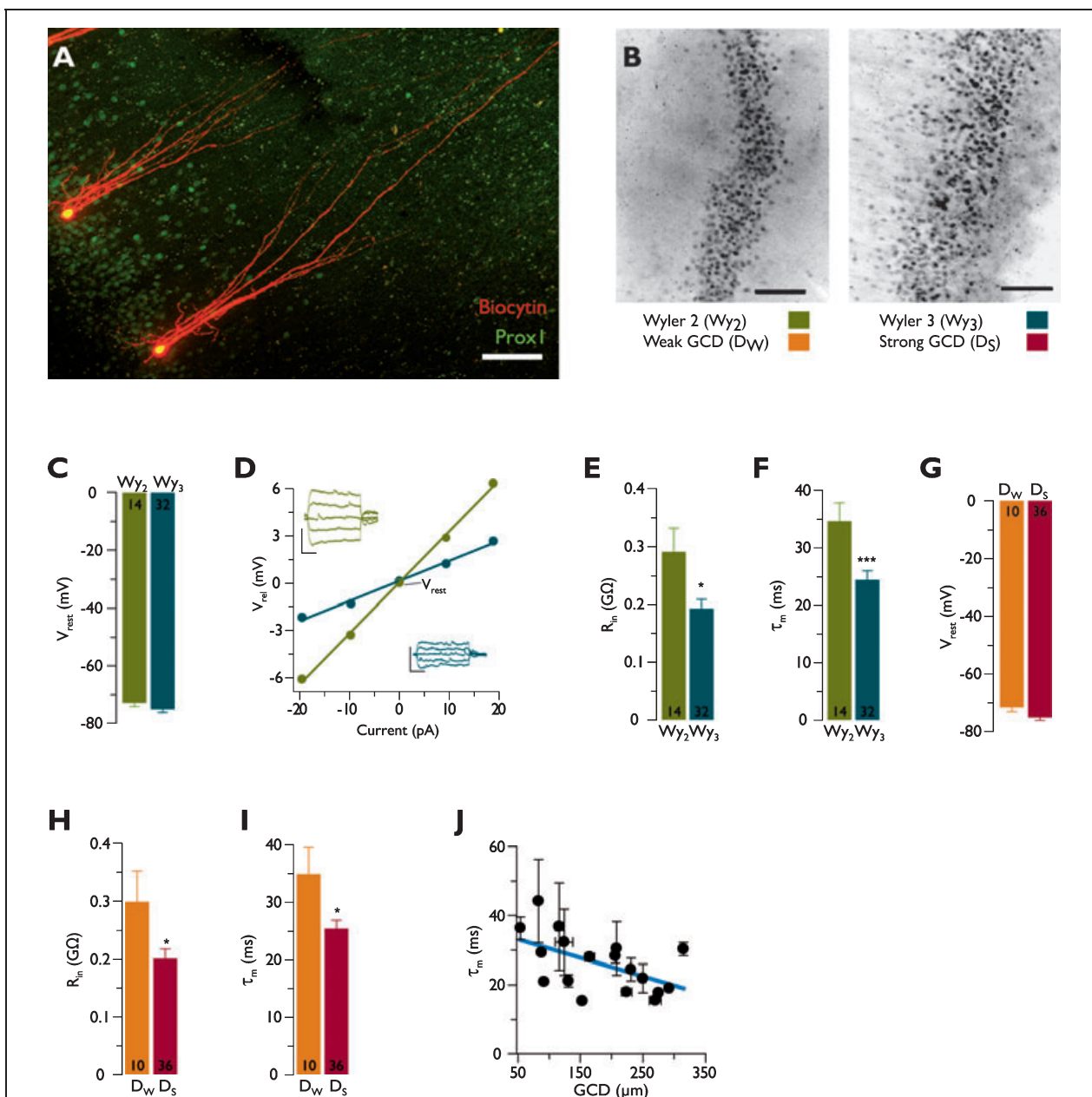
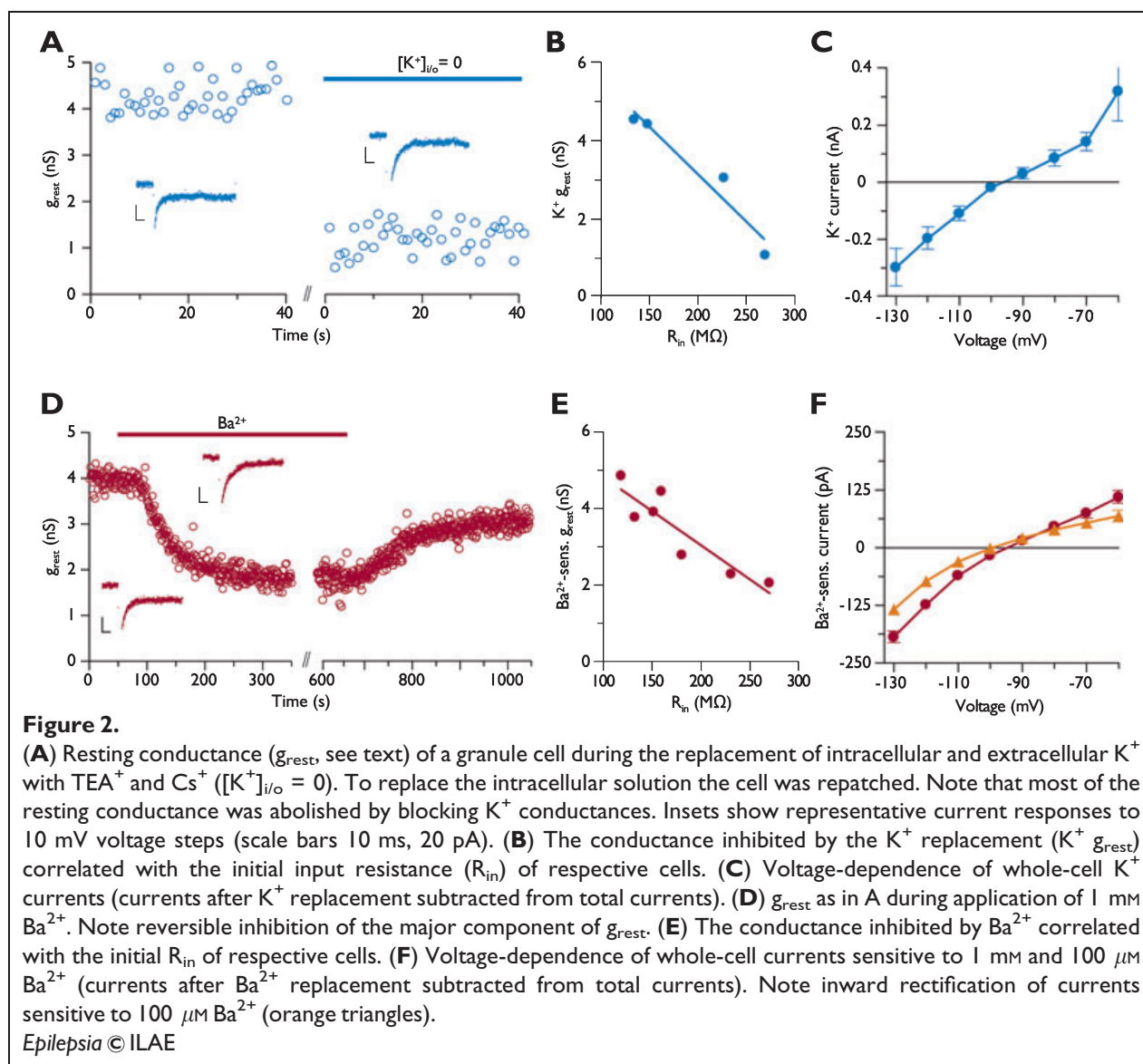


Figure 1.

(A) Examples of biocytin-labeled dentate gyrus cells of a patient with temporal lobe epilepsy (TLE). Cells were colabeled with the granule cell marker Prox1. Scale bar, 100 μ m. (B) Representative Prox1 stainings of the two patient groups into which electrophysiologic data was divided (see below): low-grade lesions, that is, Wyler grade 2–2.5 (Wy₂) with weak GCD (D_W) and high-grade lesions (3–4, Wy₃) with strong GCD (D_S). Scale bar, 100 μ m. (C) No difference was detected in resting membrane potentials (V_{rest}) of granule cells grouped according to severity of Ammon's horn sclerosis (AHS). (D) The voltage responses of Wy₃ granule cells were smaller than those of the Wy₂ granule cells (blue and green, respectively, with identical 10 pA step current stimulation). V_{rel} , voltage relative to V_{rest} . Inset scale bars, 0.4 s and 5 mV. (E–F) Input resistances (E, R_{in}) calculated from current–voltage relations as in D and membrane time constants (F, τ_m) were smaller in Wy₃ than in Wy₂ granule cells. (G–I) Analysis as in C–F, with granule cells grouped depending on the degree of GCD (see B). D_S granule cells had lower E, R_{in} (H) and τ_m values (I) than D_W granule cells. (J) The τ_m correlated with the degree of GCD of the respective samples. Numbers in bars are cells and values represent mean \pm SEM (standard error of the mean).

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also correlated with the R_{in} (Fig. 2E) ($r = 0.90$, $n = 7$, $p < 0.01$). Ba²⁺-sensitive currents showed inward rectification (Fig. 2F, circles), which became more obvious when lower Ba²⁺ concentrations were used (Fig. 2F, triangles) (100 μ M, $n = 3$).

In summary, these results show that with increasing GCD and severity of AHS, a leak conductance was increased in granule cells of TLE patients. The unchanged V_m and the pharmacologic profile indicate that K⁺ channels mediated most of this pathologic leak conductance.

DISCUSSION

We investigated the functional membrane properties of granule cells in relation to the severity of AHS and GCD, neuropathologic hallmarks often associated with TLE.

Our main conclusion is that the passive properties of granule cells are fundamentally altered depending on the severity of the sclerosis and disorganization of the granule layer. With increasing GCD, these neurons displayed a decreasing R_{in} and τ_m , both important parameters for post-synaptic signal integration. No such dispersion-related decrease in the R_{in} of granule cells had been shown in previous patch-clamp studies of hippocampi resected from TLE patients with variable degrees of AHS (Williamson et al., 1995; Isokawa, 1996; Selke et al., 2006), a disparity possibly due to methodologic differences. Because true controls of human granule cells are not available for patch-clamp recordings it is difficult to estimate their physiologic R_{in} . Whether or not the low-grade lesion group is considered “control-like” does not appear to affect our main conclusion. Most patch-clamp studies

using TLE animal models (e.g., kindling, systemic pilocarpine, or kainate injections) have described the R_{in} of granule cells as unchanged (Mody et al., 1992; Okazaki et al., 1999; Dietrich et al., 2005; Isokawa, 1996). However, most classical rat TLE models show only little GCD, and granule cells may possess different properties in these models. No comparable study has been completed so far in the intrahippocampal kainate model that does display GCD (Suzuki et al., 1995; Kralic et al., 2005; Heinrich et al., 2006).

The measurements of AHS and GCD may be subject to variability depending on factors such as patient heterogeneity and cutting angle of hippocampal specimen. However, in order to compromise our conclusion the cutting angle would have to be biased in a systematic manner correlating with the R_{in} of the cells, which we consider an unlikely scenario. The granule cell layer has a natural variation in the width, and R_{in} or τ_m could also correlate with this “physiologic GCD.” However, in mice we did not detect such a correlation (data not shown, $n = 21$ slices with 29 cells, $p > 0.6$, respectively), indicating that the increased leak is indeed specific to the pathologic situation. Increased neurogenesis has been reported in animal models without GCD (Parent et al., 1997; Scharfman et al., 2000). However, no increased neurogenesis has been detected in TLE in humans (Mathern et al., 2002; Fahrner et al., 2007) and immature neurons are easily discernable from mature granule cells (Schmidt-Hieber et al., 2004). Therefore, it is unlikely that we accidentally recorded from newborn granule cells.

Our results show that a major part of the membrane leak conductance controlling the V_m and R_{in} of human granule cells was due to a Ba^{2+} -sensitive K^+ conductance. We did not yet identify a specific ion channel for this leak conductance. The fact that V_m values were not different in the compared groups and our K^+ replacement results points to K^+ channels. Especially, certain members of the inwardly rectifying K^+ channel and two-pore K^+ channel families are consistent with the Ba^{2+} sensitivity and inward rectification in our experiments (Patel & Honore, 2001; Kubo et al., 2005). However, at this stage neither a contribution of voltage-gated or calcium-activated K^+ channels (Beck et al., 1997) nor of hyperpolarization-activated cation channels (Bender et al., 2003) can be ruled out with certainty. To prevent misinterpretations, we stress that we do not infer a causal relationship between GCD and the increased K^+ leak of granule cells.

In summary, we have shown that granule cells are “leaky” with increasing severity of TLE symptoms. This leakiness is a property suitable to shunt synaptic input (Staley & Mody, 1992) and thereby to protect granule cells from excitotoxic effects of seizure activity. Thus our results are compatible with a scenario in which seizures trigger the expression of different neuroprotective reac-

tions, including the upregulation of K^+ -channel density, which might constitute both a neuroprotective and anti-convulsive strategy in TLE.

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We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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REFERENCES

- Beck H, Clusmann H, Kral T, Schramm J, Heinemann U, Elger CE. (1997) Potassium currents in acutely isolated human hippocampal dentate granule cells. *J Physiol* 498(Pt 1):73–85.
- Ben-Ari Y. (1985) Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience* 14:375–403.
- Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. (2003) Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. *J Neurosci* 23:6826–6836.
- Blumcke I, Thom M, Wiestler OD. (2002) Ammon’s horn sclerosis: a maldevelopmental disorder associated with temporal lobe epilepsy. *Brain Pathol* 12:199–211.
- Boullier V, Schwaller B, Schurmans S, Celio MR, Fritschy JM. (2000) 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* 97:47–58.
- Brickley SG, Revilla V, Cull-Candy SG, Wisden W, Farrant M. (2001) Adaptive regulation of neuronal excitability by a voltage-independent potassium conductance. *Nature* 409:88–92.
- Dietrich D, Podlogar M, Ortman G, Clusmann H, Kral T. (2005) Calbindin-D28k content and firing pattern of hippocampal granule cells in amygdala-kindled rats: a perforated patch-clamp study. *Brain Res* 1032:123–130.
- Dudek FE, Obenaus A, Schweitzer JS, Wuarin JP. (1994) Functional significance of hippocampal plasticity in epileptic brain: electrophysiological changes of the dentate granule cells associated with mossy fiber sprouting. *Hippocampus* 4:259–265.
- Fahrner A, Kann G, Flubacher A, Heinrich C, Freiman TM, Zentner J, Frotscher M, Haas CA. (2007) Granule cell dispersion is not accompanied by enhanced neurogenesis in temporal lobe epilepsy patients. *Exp Neurol* 203:320–332.
- Haas CA, Dudek O, Kirsch M, Huszka C, Kann G, Pollak S, Zentner J, Frotscher M. (2002) Role for reelin in the development of granule cell dispersion in temporal lobe epilepsy. *J Neurosci* 22:5797–5802.
- Harvey BD, Sloviter RS. (2005) Hippocampal granule cell activity and c-Fos expression during spontaneous seizures in awake, chronically epileptic, pilocarpine-treated rats: implications for hippocampal epileptogenesis. *J Comp Neurol* 488:442–463.
- Heinemann U, Beck H, Dreier JP, Ficker E, Stabel J, Zhang CL. (1992) The dentate gyrus as a regulated gate for the propagation of epileptiform activity. *Epilepsy Res Suppl* 7:273–280.
- Heinrich C, Nitta N, Flubacher A, Müller M, Fahrner A, Kirsch M, Freiman T, Suzuki F, Depaulis A, Frotscher M, Haas CA. (2006) Reelin deficiency and displacement of mature neurons, but not neurogenesis,

- underlie the formation of granule cell dispersion in the epileptic hippocampus. *J Neurosci* 26:4701–4713.
- Houser CR. (1990) Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Res* 535:195–204.
- Isokawa M. (1996) Decrement of GABAA receptor-mediated inhibitory postsynaptic currents in dentate granule cells in epileptic hippocampus. *J Neurophysiol* 75:1901–1908.
- Jefferys JG. (1999) Hippocampal sclerosis and temporal lobe epilepsy: cause or consequence? *Brain* 122(Pt 6):1007–1008.
- Kralic JE, Ledergerber DA, Fritschy JM. (2005) Disruption of the neurogenic potential of the dentate gyrus in a mouse model of temporal lobe epilepsy with focal seizures. *Eur J Neurosci* 22:1916–1927.
- Kubo Y, Adelman JP, Clapham DE, Jan LY, Karschin A, Kurachi Y, Lazdunski M, Nichols CG, Seino S, Vandenberg CA. (2005) International Union of Pharmacology. LIV. Nomenclature and molecular relationships of inwardly rectifying potassium channels. *Pharmacol Rev* 57:509–526.
- Leblond J, Krnjevic K. (1989) Hypoxic changes in hippocampal neurons. *J Neurophysiol* 62:1–14.
- Liu M, Pleasure SJ, Collins AE, Noebels JL, Naya FJ, Tsai MJ, Lowenstein DH. (2000) Loss of BETA2/NeuroD leads to malformation of the dentate gyrus and epilepsy. *Proc Natl Acad Sci U S A* 97:865–870.
- Magloczky Z, Halasz P, Vajda J, Czirjak S, Freund TF. (1997) Loss of Calbindin-D28K immunoreactivity from dentate granule cells in human temporal lobe epilepsy. *Neuroscience* 76:377–385.
- Margerison JH, Corsellis JA. (1966) Epilepsy and the temporal lobes. A clinical, electroencephalographic and neuropathological study of the brain in epilepsy, with particular reference to the temporal lobes. *Brain* 89:499–530.
- Mathern GW, Leiphart JL, De Vera A, Adelson PD, Seki T, Neder L, Leite JP. (2002) Seizures decrease postnatal neurogenesis and granule cell development in the human fascia dentata. *Epilepsia* 43(Suppl 5):68–73.
- Mody I, Kohr G, Otis TS, Staley KJ. (1992) The electrophysiology of dentate gyrus granule cells in whole-cell recordings. *Epilepsy Res Suppl* 7:159–168.
- Naegele JR. (2007) Neuroprotective strategies to avert seizure-induced neurodegeneration in epilepsy. *Epilepsia* 48(Suppl 2):107–117.
- Nagerl UV, Mody I, Jeub M, Lie AA, Elger CE, Beck H. (2000) 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* 20:1831–1836.
- Neher E. (1992) Correction for liquid junction potentials in patch clamp experiments. *Methods Enzymol* 207:123–131.
- Okazaki MM, Molnar P, Nadler JV. (1999) Recurrent mossy fiber pathway in rat dentate gyrus: synaptic currents evoked in presence and absence of seizure-induced growth. *J Neurophysiol* 81:1645–1660.
- Olney JW, Fuller T, De Gubareff T. (1979) Acute dendrotoxic changes in the hippocampus of kainate treated rats. *Brain Res* 176:91–100.
- Parent JM, Yu TW, Leibowitz RT, Geschwind DH, Sloviter RS, Lowenstein DH. (1997) Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *J Neurosci* 17:3727–3738.
- Patel AJ, Honore E. (2001) Properties and modulation of mammalian 2P domain K+ channels. *Trends Neurosci* 24:339–346.
- Remy S, Gabriel S, Urban BW, Dietrich D, Lehmann TN, Elger CE, Heinemann U, Beck H. (2003) A novel mechanism underlying drug resistance in chronic epilepsy. *Ann Neurol* 53:469–479.
- Scharfman HE, Goodman JH, Sollas AL. (2000) Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: functional implications of seizure-induced neurogenesis. *J Neurosci* 20:6144–6158.
- Schmidt-Hieber C, Jonas P, Bischofberger J. (2004) Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* 429:184–187.
- Selke K, Muller A, Kukley M, Schramm J, Dietrich D. (2006) Firing pattern and calbindin-D28k content of human epileptic granule cells. *Brain Res* 1120:191–201.
- Sloviter RS. (1991) Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the “dormant basket cell” hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus* 1:41–66.
- Sloviter RS. (1994) The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. *Ann Neurol* 35:640–654.
- Staley KJ, Mody I. (1992) Shunting of excitatory input to dentate gyrus granule cells by a depolarizing GABAA receptor-mediated postsynaptic conductance. *J Neurophysiol* 68:197–212.
- Suzuki F, Junier MP, Guilhem D, Sorensen JC, Onteniente B. (1995) Morphogenetic effect of kainate on adult hippocampal neurons associated with a prolonged expression of brain-derived neurotrophic factor. *Neuroscience* 64:665–674.
- Tauk DL, Nadler JV. (1985) Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. *J Neurosci* 5:1016–1022.
- Thom M, Sisodiya SM, Beckett A, Martinian L, Lin WR, Harkness W, Mitchell TN, Craig J, Duncan J, Scaravilli F. (2002) Cytoarchitectural abnormalities in hippocampal sclerosis. *J Neuropathol Exp Neurol* 61:510–519.
- Wickenden AD. (2002) Potassium channels as anti-epileptic drug targets. *Neuropharmacology* 43:1055–1060.
- Williamson A, Telfeian AE, Spencer DD. (1995) Prolonged GABA responses in dentate granule cells in slices isolated from patients with temporal lobe sclerosis. *J Neurophysiol* 74:378–387.
- Wyler AR, Dohan FC, Schweitzer JB, Berry AD. (1992) A grading system for hippocampal sclerosis. *J Epilepsy* 5:220–225.
- Zentner J, Hufnagel A, Wolf HK, Ostertun B, Behrens E, Campos MG, Solymosi L, Elger CE, Wiestler OD, Schramm J. (1995) Surgical treatment of temporal lobe epilepsy: clinical, radiological, and histopathological findings in 178 patients. *J Neurol Neurosurg Psychiatry* 58:666–673.