Axosomatic Synapses on Granule Cells are Preserved in Human Non-infiltrating Tumour or Lesion-related and Mesial Temporal Sclerotic Epilepsy, but Markedly Reduced in Tumour-infiltrated Dentate Gyrus with or without Epilepsy

Seress L.[1], Ábrahám H.[1], Dóczi T.[2], Pokorny J.[3], Klemm J.[4], Bakay R. A.[4]
[1]Central Electron Microscopic Laboratory of Faculty of Medicine, University of Pécs, Hungary;
[2]Department of Neurosurgery of Faculty of Medicine, University of Pécs, Hungary;
[3]Institute of Physiology of the First Faculty of Medicine, Charles University in Prague, Czeh Republic;
[4]Department of Neurosurgery, Rush Presbyterian-St. Luke's Medical Center, Chicago, Illinois, USA

Received December 2, 2004, Accepted December 29, 2004

Abstract: Granule cells of the human hippocampal dentate gyrus were examined. In controls, granule cells displayed somatic spines and cell nuclei with small infoldings. In addition, the cytoplasm of human granule cells always displayed lipofuscin. Subsurface cisterns of endoplasmic reticulum were frequently observed in the human granule cells. Two types of axosomatic synapses were found; most frequently symmetric and less frequently asymmetric. Many of the axosomatic synapses were isolated by glial processes in tumour or lesion-related epileptic patients, but the ultrastructural characteristics of granule cells were not different from those of the control patients. Large bundles of reactive astroglial fibres appeared regularly in all layers of the dentate gyrus. In tumour infiltrated hippocampi, glial processes dominated the neuropil and the number of perisomatic synapses was markedly reduced. Reduction in the number of perisomatic synapses did not correlate with severity and duration of seizures but did correlate with the malignancy of the tumour. It is suggested that reduction of perisomatic inhibition may not be a characteristic of granule cells in the epileptic human dentate gyrus.

Key words: Hippocampus – Perisomatic inhibition – Epilepsy – Neuropathology.

Supported by the Hungarian Scientific Research Fund (OTKA) grant #T047109.

Mailing address: Prof. László Seress, MD., PhD., Central Electron Microscopic Laboratory, Faculty of Medicine, University of Pécs, Szigeti u. 12., 7643 Pécs, Hungary, Phone: +36 72 536 060, e-mail: laszlo.seress@aok.pte.hu

Introduction

It is generally accepted that the axon terminals form exclusively symmetric synapses with the somata of glutamatergic pyramidal cells of the cerebral cortex [1, 2], whereas on the cell bodies of GABAergic local circuit neurones both symmetric and asymmetric synapses occur [3, 4]. Although, this general principle is valid for the archicortical hippocampal formation, there is one exception. Granule cells of the dentate gyrus are glutamatergic but display both symmetric and asymmetric axosomatic synapses [5]. Granule cells of the dentate gyrus form a homogeneous group in rodents, but they display significant variability in the primate hippocampus [6]. When compared to rodents, granule cells of non-human primates display a low number of axosomatic synapses [7]. Parvalbumin-positive basket cells, which provide the main inhibitory synaptic input for the somata of granule cells, are low in number in the monkey and human dentate gyrus [8, 9, 10]. Similarly, the number of perisomatic symmetric synapses is similarly low in the human as it is in the monkey [11].

Perisomatic inhibition is regarded to be crucial for defending the cell from overexcitation and a decrease of perisomatic GABAergic inhibition was considered as a cause of focal epilepsy in rodent animal models [12]. A decreased number of perisomatic inhibitory synapses may be considered to be pathognomic for human epilepsy, although a recent study demonstrated an unchanged number of perisomatic synapse for the dentate granule cells in mesial temporal sclerotic epilepsy [11].

In the present study, the frequency and distribution of axosomatic synapses as well as the ultrastructural characteristics of granule cells are described in the human hippocampal dentate gyrus of controls and epileptic patients.

Materials and Methods

Human "control" hippocampal tissue was obtained from three cases (Table 1). In cases 1 and 3, coroner's autopsy was performed and brains were removed two hours after death. The brain was perfused through the carotid arteries first with physiological saline followed by a fixative containing 4% paraformaldehyde. In control case 2, the hippocampus was placed into the 4% paraformaldehyde fixative immediately after the surgical removal. From all control hippocampi, 10-mm-thick slices were cut and postfixed with 2.5% glutaraldehyde for 2h at room temperature. Then blocks were treated as described below for the surgically removed tissue.

Axosomatic synaptic density on granule cells of mesial temporal sclerotic epilepsy patients (Tab. 1) was examined on electron microscopic preparations obtained and previously described by Franck and co-workers (12). Patients had intractable mesial temporal epilepsy with different degrees of hippocampal atrophy from mild gliosis (case 1161) to hippocampal sclerosis (cases 1225, 1227). In the previous study, only the biocytin-filled granule cells were examined and synapses

were identified only on the labelled granule cells. In the present study, axosomatic synaptic coverage of randomly selected well-preserved non-labelled granule cells was examined inside the granule cell layer.

Axosomatic synaptic density on granule cells of tumour-related and tumour-infiltrated epileptic patients (Tab. 1) was counted in preparations obtained from surgically removed hippocampi. The growing tumours of the temporal neocortex either compressed the hippocampal region and caused herniation of the uncus or infiltrated the hippocampus. Mild or moderate gliosis was observed, but hippocampal sclerosis was not found in these cases.

The surgically removed brain tissue was immersed in 4% paraformaldehyde buffered with phosphate buffer (PB, 0.1 M, pH 7.4). After transportation to the histological laboratory, the hippocampi were cut in 10-mm-thick blocks immersed in 4% paraformaldehyde and kept for 2-4 h at room temperature. Then the blocks were cut with Vibratome at 60 or 80 μm. Sections were placed in 2.5% glutaraldehyde solution for 2h at room temperature, washed several times with PB, osmicated with 1% OsO_4 for 1 h, dehydrated and flat-embedded in Durcupan according to routine electron microscopic procedures. Flat embedded sections were examined under the light microscope. Areas of interest were selected and re-blocked in Durcupan. Thin sections were collected on Parlodion-coated one-slot grids, and stained with lead citrate and uranyl acetate. Sections were examined with a JEOL 1200 electron microscope. A continuous row of 80–120 granule cells could be examined on each one-slot grid in each of the thin section. A goniometer was used to tilt the grids for the identification of synapses as well as to determine the type of synapses. When a synapse (synaptic density, vesicle accumulation,

Table 1

Group	Case	Age (years)	Gender	Diagnose	Seizure onset
Control	Control 1.	47	♂	cardiac arrest	no seizure
	Control 2.	48	♂	lung cancer with intracranial metastasis	no seizure
	Control 3.	65	♂	cardiac arrest	no seizure
Mesial temporal epipeltic cases	JF 1161	43	♀	CPS	23 years
	JF 1227	43	♀	SPS, CPS	28 years
	JF 1225	44	♀	CPS	34 years
Tumour and lesion-related epileptic cases	Case No. 11.	45	♀	meningeoma	1.5 years
	Case No. 12.	46	♂	glioblastoma multiforme	6 months
	Case No. 17.	19	♂	angioma	3 years
	Case No. 18.	61	♂	glioblastoma multiforme	6 months
Tumour-infiltrated hippocampus	Case No. 1	65	♀	glioblastoma	no seizure
	Case No. 28	17	♀	glioma	2 years
	Case No. 15	11	♂	glioblastoma	no seizure
	TU/00/4	50	♂	glioma	4 months

Abbreviations: SPS, simple partial seizure; CPS, complex partial seizure

synaptic cleft) could not equivocally be identified than the apposing terminal was not counted in this study.

Results

The granule cell layer of the control human dentate gyrus was densely packed with granule cells (Fig. 1A and B). Somata of granule cells directly apposed each other without having intervening glial processes (Fig. 2A). The thin cytoplasm frequently contained lipofuscin pigment deposits, but never formed large aggregates (Fig. 2A and B). Somal spines (Fig. 2C) and small nuclear indentations were commonly found in granule cells. Somal spines varied in both diameter and length, but lacked polyribosomes or granular endoplasmic reticulum. Subsurface cisterns were frequently observed under the plasma membrane and they preferentially occurred in the vicinity of synaptic terminals (Fig. 2D). Somata of granule cells were apposed by axon terminals that formed symmetric (Fig. 1C) or asymmetric synapses (Fig. 1D). The average number of symmetric axosomatic synapses per granule cell per thin section was 0.44 (Tab. 2). The number of synapses found on a single granule cell soma per thin section ranged from 0 to 3. About 65% of granule cells displayed no synapses on their somal surface in the examined thin section. The percentage of asymmetric axosomatic synapses was 6–10% (Tab. 2) and most of them were on granule cells located at the border of the molecular layer.

Diameter of somata of granule cells could be measured from semithin section in the light microscope (Fig. 1B) and also from thin sections in the electron microscope using a calibration bar drawn on the screen of the electron microscope. A population of at least 100 neurones was measured in the electron microscope for each case except for the mesial temporal sclerotic epileptic cases, where the quality of sections did not allow the examination of such a large population, because sections were prepared from tissue slices. The mean of shorter and longer diameters was calculated in those cases where the soma was elliptical. From the diameter, the circumference of the soma was calculated. For example, in control case No.1, the average length of somatic membrane for a granule cell was 39.72 μm. The length was calculated from the average of somal diameters that was 12.65 $\pm$ 0.135 μm (S.E.M.) as measured in the electron microscope. The average nuclear diameter was 9.5 $\pm$ 0.138 μm (S.E.M.) and the nucleoli were 2 μm long in diameter. In average, the synaptic density was 1 synapse for 85–100 μm long somal membrane.

In mesial temporal sclerotic epileptic cases there was no significant difference in the number or size of the axosomatic synapses (Tab. 3) when compared to the controls. The somal features of granule cells including the lipofuscin deposits and submembrane cisterns were also similar as found in the controls. Astroglial processes were more frequent in mesial temporal epileptic cases than in controls, but glial processes did not separate granule cells.

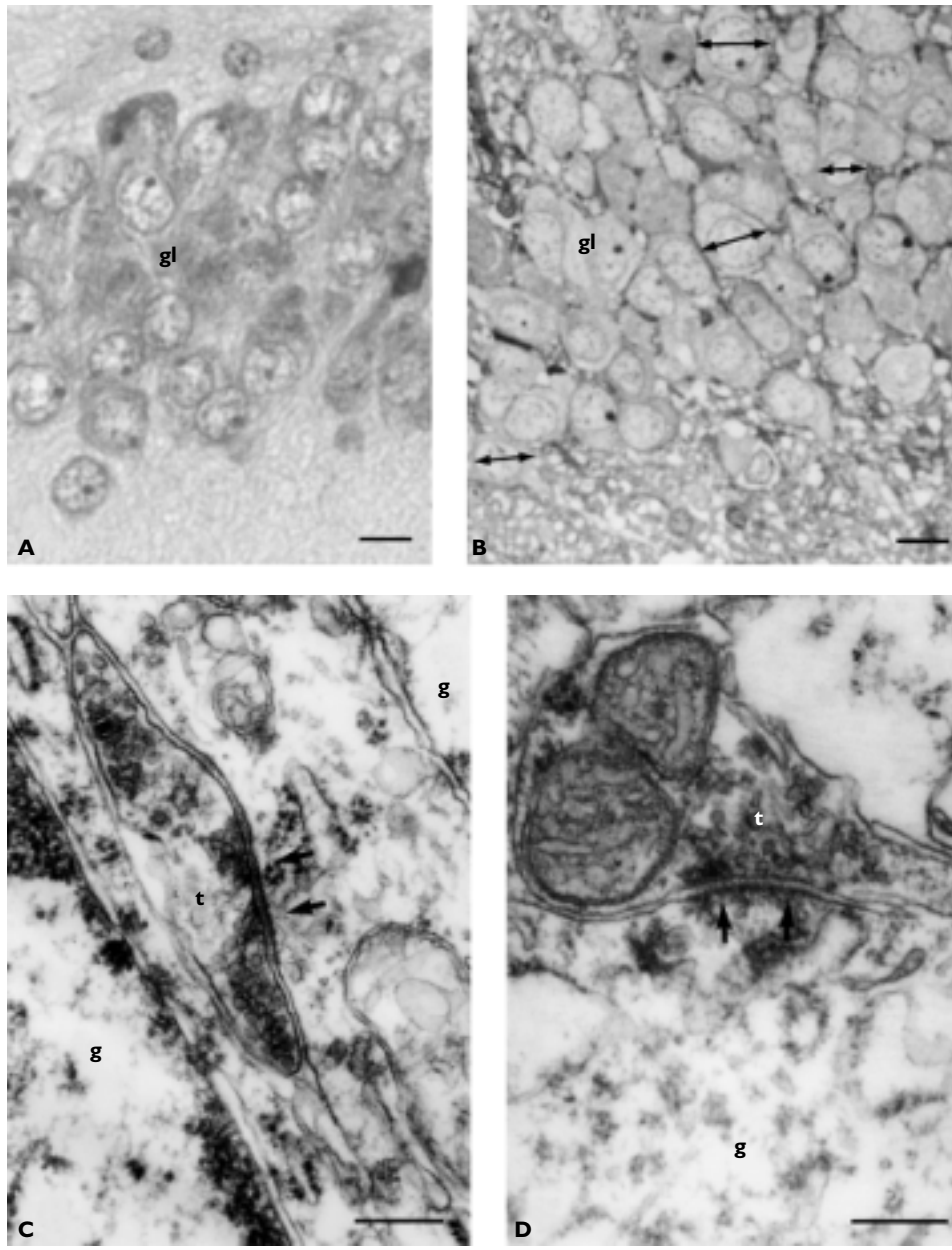

*Figure 1 – **A**. Photomicrograph of cresyl violet stained 10-μm-thick section of the granule cell (gl) layer and **B**. Photomicrograph of toluidine blue stained semi-thin section of the granule cell layer (gl) from the hippocampus of control case 2. Double-headed arrows indicate the somatic diameter as it was measured to calculate the length of somatic perimeter. **C** and **D.** Electron micrographs of axon terminals (t) that form symmetric (**C**) or asymmetric (**D**) synapses (arrows) with somata of granule cells (g) in the dentate gyrus of control case No.1. Bar = 10 μm for A and B; 0.2 μm for C and D.*

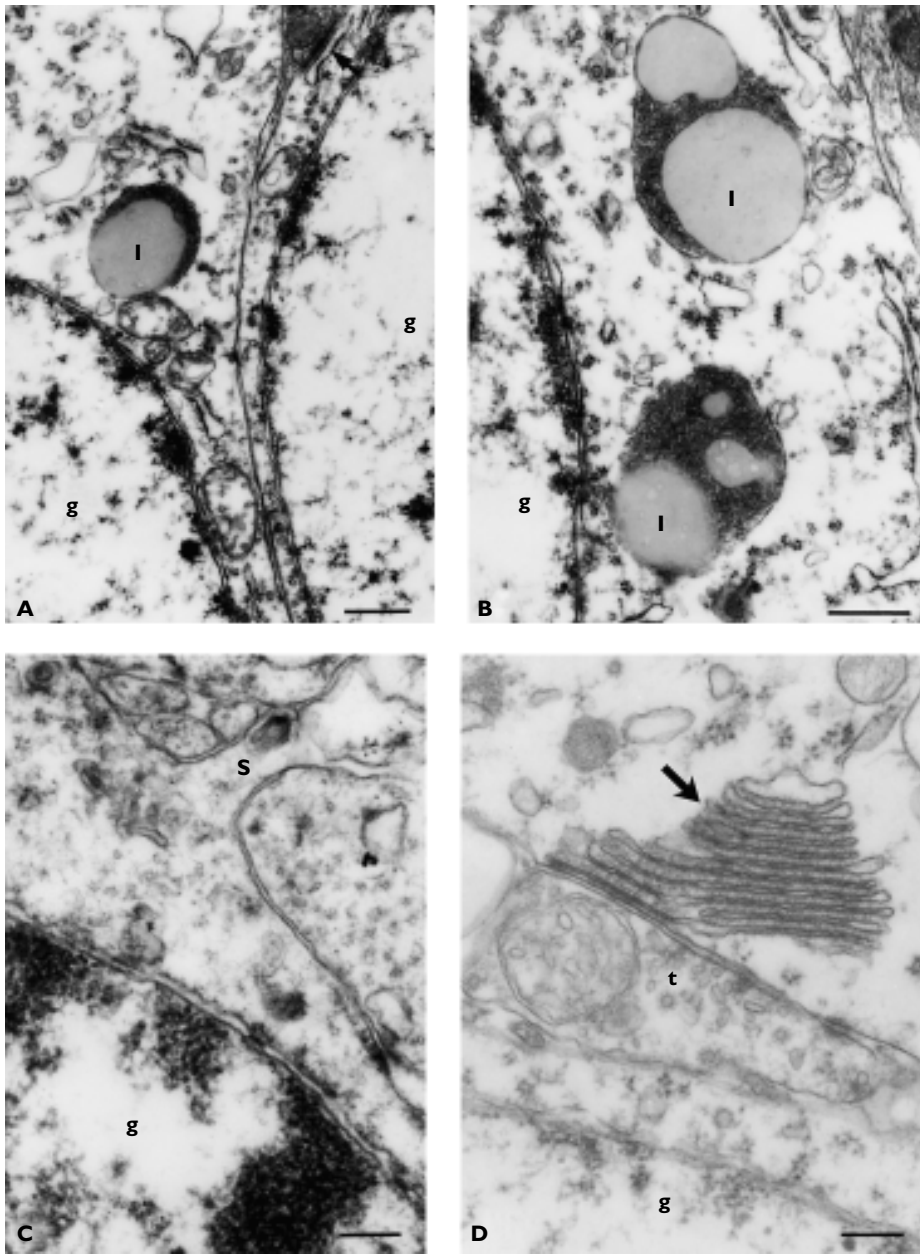

Figure 2 – Electron micrographs of different features of granule cells (Control case No.1). ***A.*** *Apposing somata of granule cells (g) without having glial sheets among them. The cytoplasm (g) contains lipofuscin (l).* ***B.*** *Multiple large lipofuscin deposits (l) regularly appear in granule cells.* ***C.*** *Granule cells (g) regularly display somal spines (s) and well developed subsurface cisterns (arrow) that appear to be continuous with the endoplasmic reticulum (**D**). These cisterns frequently locate in the vicinity of synapses or where an axon terminal (t) apposes the soma. Bar = 0.5 μm for A and B and 0.2 μm for C and D.*

In the non-infiltrating lesion or tumour-related cases (Tab. 1) the neocortical tumours did not infiltrate the hippocampus as was verified by the pathological examination. The growing tumours caused life-threatening compression of the neighbour brain tissue including the hippocampal formation. In all cases, seizures of variable duration were recorded (Tab. 1). Independent of the length of epileptic history before the operation, perisomatic innervation of granule cells of the surgically removed hippocampal dentate gyrus did not differ from that of the control. Frequency, shape and size of axon terminals were similar as found in the controls (Tab. 4, Fig. 4A). However, the granule cell layer was loosely packed with granule cells (Fig. 3A) and glial processes were frequent among granule cells (Fig. 4A). Astroglial processes surrounded axon terminals, but did not disrupt the synaptic contact (Fig. 4A). The abundant filament content of astroglial processes suggests that all of these processes belonged to activated glial cells. The majority of glial processes run perpendicular to the granule cell layer and most of the somata of activated glial cells were in the hilus. Separation of somata of granule cells varied considerably inside the same granule cell layers, because in some places only small profiles (1μm) of cross-sections of glial processes appeared, whereas a few hundred microns away, somata of other granule cells were completely separated by glial fiber bundles (Fig. 4A).

In the tumour-infiltrated cases, all layers of dentate gyrus were occupied by tumour cells (Fig. 3B). As a result, granule cells were separated not only by glial processes, but also by the somata of tumour cells. Neuropil of the dentate gyrus was disrupted by the glial processes forming thick bundles among granule cells (Fig. 4B). Correspondingly, the number of axosomatic synapses was low in all of these cases, especially in the heavily infiltrated cases (Fig. 3B, 4B) where the number of synapses was one-tenth of the control value (Tab. 5). Interestingly, the low number of synapses did not correlate with seizure frequency, because similarly low numbers were found in those cases where no seizures were recorded (Tab. 1).

Discussion

The results of this study suggest that human granule cells display characteristics similar to those of the non-human primate in respect of their somal spines, nuclear infoldings and the number of axosomatic synapses [7]. In addition, a commonly

Table 2 – Number of axosomatic synapses per granule cell in controls

	Number of cells	Number of synapses (Number of terminals forming synapses)	Synapses / cell Mean ± S.E.M.	Percentage of asymmetric synapses (%)
Control 1.	243	112 (108)	0.46 ± 0.044	8
Control 2.	235	87 (84)	0.38 ± 0.053	6
Control 3.	190	90 (89)	0.47 ± 0.075	10

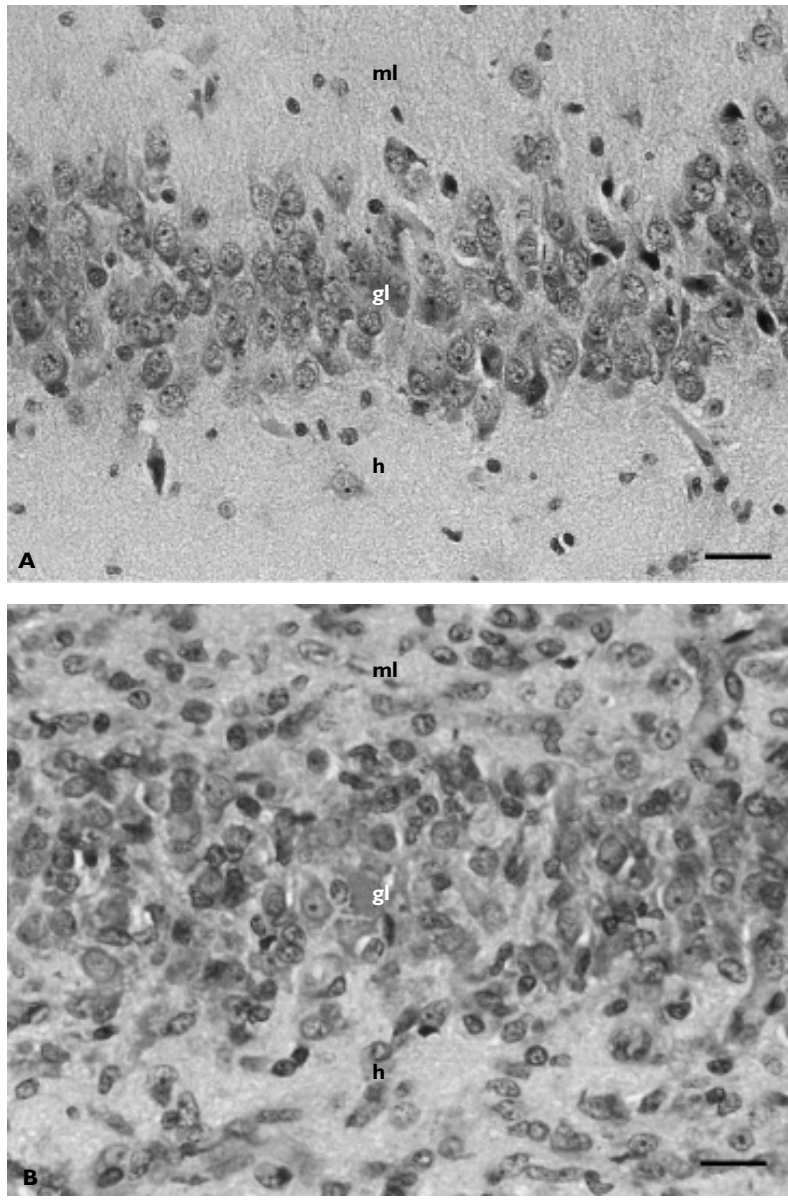

Figure 3 – Light microscopic photomicrographs of the granule cell layer in epileptic patients. ***A.*** *Granule cells are not as closely packed in the epileptic granule cell layer (gl) of the dentate gyrus as in controls (compare with A and B on Fig. 1). The molecular layer (ml) and hilus (h) has the regular density of cells similarly as seen in controls. A tumour-related case of epilepsy, where a neocortical meningeoma compressed the hippocampal region.* ***B.*** *The granule cell layer (gl) as well as the molecular layer (ml) and hilus (h) are densely packed by tumour cells. The space among granule cells is large therefore the cellular density in granule cell layer (gl) appears to be much smaller than in controls. Dentate gyrus of an epileptic patient where the ganglioglioma infiltrated the hippocampus. Bar = 25 μm for A and B.*

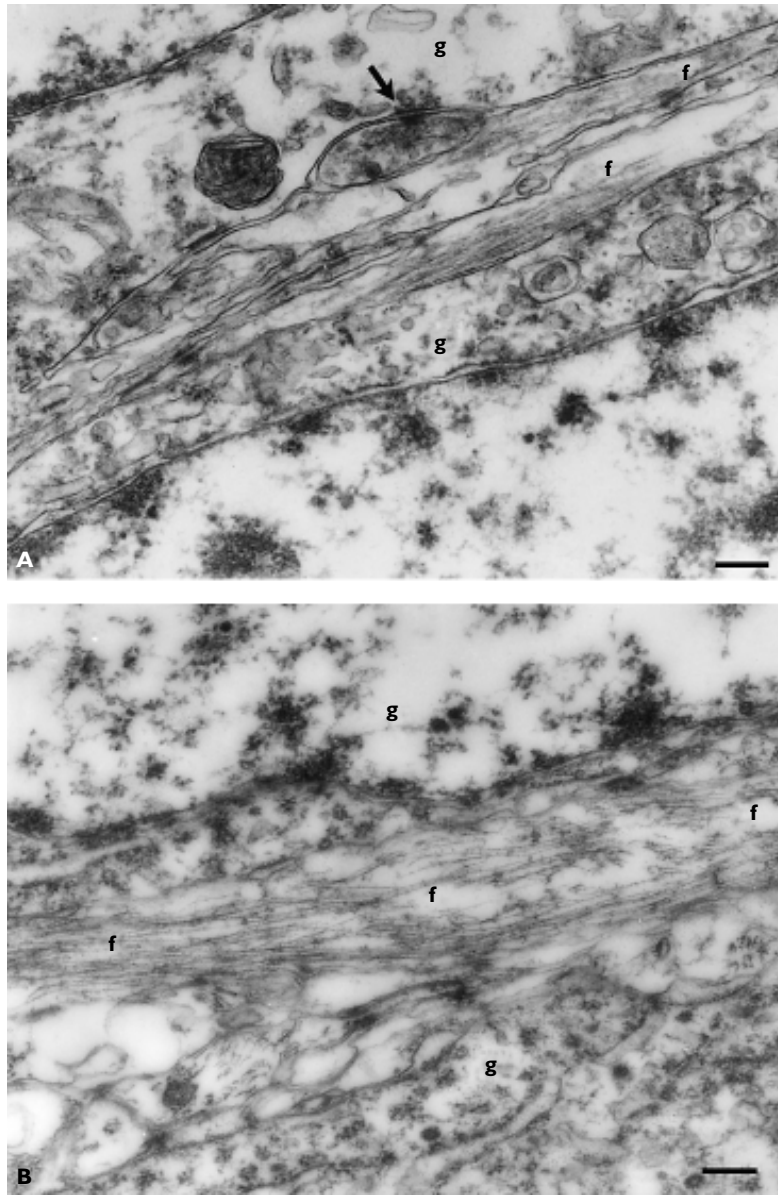

Figure 4 – Electron micrographs of the granule cell layer from epileptic patients. ***A.*** *Granule cells (g) are separated by glial processes (f). The glial fibres appear to isolate axon terminals (t) but do not destroy them in the tumour-related epilepsy. Same case as shown on Fig. 3A.* ***B.*** *Wide bands of glial processes (f) separate granule cells (g) in the dentate gyrus of epileptic patient where the tumour infiltrated the dentate gyrus. Same case as shown on Fig. 3B. Bar = 0,5 μm for A and B.*

observed feature of human granule cells was the occurrence of lipofuscin pigment granules. Lipofuscin has been described for the human pyramidal and non-pyramidal hippocampal neurones, but not for granule cells [14, 15]. It appears that in human lipofuscin occurs in all hippocampal neurones and the size, location and amount of pigment granules are characteristic for the neuronal type.

Another interesting feature was the frequent occurrence of subsurface cisterns. Subsurface cisterns have been known to occur in neurone [16]. Indeed, such

Table 3 – Number of axosomatic synapses per granule cell in mesial temporal epileptic cases

	Number of cells	Number of synapses	Synapses / cell Mean ± S.E.M.	Percentage of asymmetric synapses (%)
JF 1161.	50	13	0.26 ± 0.032	–
JF 1225.	50	18	0.36 ± 0.050	5
JF 1227.	50	24	0.48 ± 0.055	8

Table 4 – Number of axosomatic synapses per granule cell in tumour-related epilepsy (Cases 11, 12, 18) and lesion-related epilepsy (Case 17)

	Number of cells	Number of synapses	Synapses / cell Mean ± S.E.M.	Percentage of asymmetric synapses (%)
Case No. 11.	100	36	0.36 ± 0.055	not counted
Case No. 12.	100	39	0.39 ± 0.062	not counted
Case No. 17.	100	46	0.46 ± 0.041	not counted
Case No. 18.	154	87	0.57 ± 0.071	7

Table 5 – Number of axosomatic synapses per granule cell in epileptic cases with tumour-infiltrated hippocampus

	Number of cells	Number of synapses	Synapses / cell Mean ± S.E.M.	Percentage of asymmetric synapses (%)
Lightly infiltrated				
Case No. 1.	50	9	0.18 ± 0.036	not counted
Case No. 28.	90	17	0.21 ± 0.032	17
Heavily infiltrated				
Case No. TU/00/4.	187	9	0.05 ± 0.004	11
Case No. 15.	107	9	0.08 ± 0.005	11

cisterns exist in pyramidal neurones of Ammon's horn, but less frequently than in granule cells. Subsurface cisterns are not characteristic for the human granule cells only, because they were also found in rodent granule cells (unpublished observation). Such cisterns are known to participate in the Ca^{++} homeostasis of the cell [17]. It is not known whether these cisterns contain calcium-binding proteins, although they have a strategic location close to synapses to accumulate free Ca^{++}.

The granule cells of the human dentate gyrus displayed a number of axosomatic synapses similar to those found in rhesus monkeys [7]. The majority of the axosomatic synapses (90%) was of symmetric type, similarly to those in monkeys [7]. A previous study demonstrated a similarly low number (1.2/cell) of axosomatic synapses on human granule cells [18]. Identification of synapses is difficult in non-perfused tissues; therefore the difference between the absolute values can be explained with the methods used. In the present study, synapses were identified directly in the microscope, whereas Wittner et al. [18] used a computer assisted high-resolution digital camera system. Neither Wittner et al. [18] nor our study could demonstrate a significant change in the average number of symmetric synapses in mesial temporal sclerotic epileptic patients. A previous finding suggested that most of axosomatic synapses on the somata of granule cells in human mesial temporal sclerotic epileptic hippocampi were of asymmetric type [12]. However, in that study only a few biocytin-labelled granule cells were examined in the electron microscope. In biocytin-labelled cells, the DAB reaction frequently interferes with a clear identification of the type of synapses. In this study, we have not found significant change in the percentage of asymmetric axosomatic synapses neither in mesial temporal sclerotic epilepsy nor in non-infiltrating tumour-related epilepsy. Our recent findings support the results of previous studies, showing no decrease in the number of GABAergic neurones and symmetric axosomatic terminals in the epileptic dentate gyrus [11, 18].

In the tumour-infiltrated dentate gyrus, the number of axosomatic synapses was markedly reduced even in those cases when the tumour infiltration was moderate, suggesting that processes of neoplastic glial cells separate axon terminals from their postsynaptic targets, whereas the reactive astroglia fibres only isolate the synaptic axon terminals. Interestingly, the reduction in axosomatic innervation did not correlate with onset of seizures, because similar loss of terminals occurred in epileptic and non-epileptic tumour-infiltrated cases. These results strengthen the view that a change in perisomatic innervation of granule cells has no direct relationship with the onset of epilepsy.

Acknowledgements

The authors wish to thank dr. Philip A. Schwartzkroin for kindly providing the electron microscopic material prepared by late JoAnn E. Franck whose helpful and kind personality will always be remembered by her former colleagues.

References

1. COLONNIER M.: Synaptic patterns of different cell types in the different laminae of the cat visual cortex. *Brain Res.* 9: p. 268–287, 1968.
2. PARNAVELAS J. G., SULLIVAN K., LIEBERMAN A. R., WEBSTER K. E.: Neurones and their synaptic organization in the visual cortex of the rat. Electron microscopy of Golgi preparations. *Cell. Tiss. Res.* 83: p. 499–517, 1977.
3. SOMOGYI P., KISVARDAY Z. F., MARTIN K. A. C., WHITTERIDGE D.: Synaptic connections of morphologically identified and physiologically characterized large basket cells in the striate cortex of cat. *Neuroscience* 10: p. 261–295, 1983.
4. HENDRY S. H. C., HOUSER C. R., JONES E. G., VAUGHN, J. E.: Synaptic organization of immuncytochemically identified GABA neurones in the monkey sensory-motor cortex. *J. Neurocytol.* 12: p. 639–660, 1983.
5. SERESS L., RIBAK C. E.: A substantial number of asymmetric axosomatic synapses is a characteristic of the granule cells of the hippocampal dentate gyrus. *Neurosci. Letters*. 56: p. 21–26, 1985.
6. SERESS L., FROTSCHER M.: Morphological variability is a characteristic feature of granule cells in the primate fascia dentata: a combined Golgi/electron microscopic study. *J. Comp. Neurol.* 293: p. 253–267, 1990.
7. SERESS L., RIBAK C. E.: Ultrastructural features of primate granule cell bodies show important differences from those of rats: axosomatic synapses, somatic spines and infolded nuclei. *Brain Res*. 569: p. 353–357, 1992.
8. RIBAK C. E., NITSCH R., SERESS, L.: Proportion of parvalbumin-positive basket cells in the GABAergic innervation of pyramidal and granule cells of the rat hippocampal formation. *J. Comp. Neurol.* 300: p. 449–461, 1990.
9. SERESS L., GULYAS A. I., FREUND, T. F.: Parvalbumin- and calbindin D28k-immunoreactive neurones in the hippocampal formation of the macaque monkey. *J. Comp. Neurol.* 313: p.162–177, 1991.
10. SERESS L., GULYAS A. I., FERRER I., TUNON T., SORIANO E., FREUND, T. F.: Distribution, morphological features, and synaptic connections of parvalbumin- and calbindin D28k-immunoreactive neurones in the human hippocampal formation. *J. Comp. Neurol.* 337: p. 208–230, 1993.
11. MAGLOCZKY, ZS., WITTNER, L., BORHEGYI, ZS., HALASZ, P., VAJDA, J. CZIRJAK, S., FREUND, T. F.: Changes in the distribution and connectivity of interneurons in the epileptic human dentate gyrus. *Neuroscience* 96: p. 7–25, 2000.
12. FRANCK J. E., POKORNY J., KUNKEL D. D., SCHWARTZKROIN P. A.: Physiologic and morphologic characteristics of granule cell circuitry in human epileptic hippocampus. *Epilepsia* 36: p. 543–558, 1995.
13. RIBAK C. E.: Epilepsy and the Cortex. In: Cerebral Cortex. Vol. 9. Peters A., Jones E.G. (eds.) Plenum Press, 1991, p. 427–483.
14. MAGLOCZKY ZS., HALASZ P., VAJDA J., CZIRJAK S., FREUND T. F.: Loss of calbindin-D28k immunoreactivity from dentate granule cells in human temporal lobe epilepsy. *Neuroscience* 76: p. 377–385, 1997.
15. OLBRICH H. G., BRAAK H.: Ratio of pyramidal cells versus non-pyramidal cells in sector CA1 of the human Ammon's horn. *Anat. Embryol.* 173: p. 105–110, 1985.
16. ROSENBLUTH J.: Subsurface cisterns and their relationship to the neuronal plasma membrane. *J. Cell Biology* 13: p. 405–421, 1962.
17. MCBURNEY R. N., NEERING I. R.: Neuronal calcium homeostasis. *TINS* 10: p. 164–169, 1987.
18. WITTNER L., MAGLOCZKY ZS., BORHEGYI ZS., HALASZ P., TOTH SZ., EROSS L, SZABO Z., FREUND T. F.: Preservation of perisomatic inhibitory input of granule cells in the epileptic human dentate gyrus. *Neuroscience* 108: p. 587–600, 2001.