

A light and electron microscopic study of oedematous human cerebral cortex in two patients with post-traumatic seizures

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Primary objective: Brain cortical biopsies of two patients with clinical diagnosis of complicated brain trauma who had seizures, were studied by means of light and electron microscopes in order to correlate structural alterations with seizure activity.

Methods and procedures: Biopsy samples of left frontal cortex and right parietal cortex were processed by current techniques for light and transmission electron microscopy.

Results: The tissue showed severe vasogenic oedema with perivascular and intraparenchymatous haemorrhages. At the capillary wall, increased vesicular and vacuolar transendothelial transport, open endothelial junctions, thickened basement membrane and swollen perivascular astrocytic end-feet were observed. Some pyramidal and non-pyramidal nerve cells appeared dense and shrunken and others exhibited marked intraneuronal enlargement of membrane compartment. The myelinated axons displayed signs of degeneration and a process of axonal sprouting. Numerous swollen asymmetrical axo-dendritic synaptic contacts were observed in the neuropil, which exhibited mostly closely aggregated spheroidal synaptic vesicles toward the presynaptic membrane and numerous exocytotic vesicles sites. The perisynaptic astrocytic ensheathment appeared retracted or absent, whereas the extracellular space appeared notably dilated. Synaptic disassembly was also observed.

Conclusion: The findings demonstrate, in two patients with post-traumatic seizure activity, brain barrier dysfunction, vasogenic oedema, anoxic-ischaemic neurons, axonal sprouting, numerous altered excitatory synapses and synaptic disassembly. Some considerations on clinical and research applications are discussed.

Introduction

Examination of surgically removed cerebral cortex in epilepsy reveals pathological changes that could account for the structural basis of clinical manifestations [1]. Ischaemic neuronal degeneration, oedema of astrocyte and tortuous capillaries with thickened, reduplicated basement membrane have been reported by Lu [2] in cryptic temporal lobe epilepsy. Epileptogenesis has been electrophysiologically recorded in cronically injured cerebral cortex [3]. A selective loss of somatostatin- and neuropeptide Y-immunoreactive neurons has been reported in rats with cerebral ischaemia and in patients with tumour and non-tumour-related temporal lobe

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epilepsy [4, 5]. Represa and Ben-Ari [6] reported mossy fibre sprouting and multiple ectopic asymmetrical synapses in the CA3 field of the hippocampus, produced by brief seizure episodes after the application of high K^+ or excitatory amino acids. Schoimair *et al.* [7] reported dendritic vacuoles and spontaneous bioelectric activity in CA3 neurons in hippocampal slices exposed to pentylenetetrazole. Swelling and degeneration of neurons have been also reported by McDonald *et al.* [8] in neonatal rats with seizures and brain injury induced by a metabotropic glutamate receptor agonist. Recurrent limbic seizures cause a dramatic increase in the number of somatic spines and asymmetric synaptic contacts [9]. Pollard *et al.* [10] reported kainate-induced apoptotic cell death in hippocampal neurons in epileptic brain damage. Salin *et al.* [11] showed a significant degree of axonal reorganization in neurons of chronic neocortical epileptogenic lesions. Degenerated dark pyramidal cells were found by Ribak and Baram [12] in CRH-induced status epilepticus in infant rats. Drakew *et al.* [13] reported spine loss in experimental epilepsy induced by bicuculine and picrotoxin in 'epileptic' slices culture of hippocampus. Friedman [14] found a selective reduction of GluR2 protein in adult hippocampal CA3 neurons following status epilepticus, but prior to cell loss. Sankar *et al.* [15] described synaptic reorganization, necrosis and apoptosis in status epilepticus induced neuronal injury by the lithium-pilocarpine model. More recently, Fujikawa *et al.* [16], using a similar model, also reported necrotic neurons with internucleosomal DNA fragmentation in adult rats. Morin *et al.* [17] reported specific changes in CA1 inhibitory interneurons in cronically hyperexcitable hippocampus. Pierce *et al.* [18] studied the subcellular distribution of peptidergic dense core vesicles in the rat hippocampal mossy fibre pathway before and after seizures. The above mentioned findings widely document the morphological changes of animal and human central nerve tissue induced by seizure activity. Most of the morphological changes described above have been reported thus far in the hippocampal formation. The aim in the present study was to examine by means of light and electron microscopy the changes of frontal, parietal and temporal cortical regions of two patients with brain trauma and seizure activity, which exhibited alterations of capillary wall, neurons, neuroglial cells, myelinated axons and axodendritic synapses.

Materials and methods

Cortical biopsies of two patients with clinical diagnosis of brain trauma were taken during neurosurgical operation and examined with light and transmission electron microscopes. The neurosurgical study was performed and the cortical biopsies were taken according to basic principles of the Helsinki Declaration.

Two-to-five millimetres thick cortical biopsies were immediately fixed in the surgical room in 4% glutaraldehyde-0.1 M phosphate or cacodylate buffer, pH 7.4, at 4°C. After a 2 hour glutaraldehyde-fixation period, the cortical biopsies were divided into ~1 mm fragments and observed under a stereoscopic microscope to check the quality of fixation of the sample, glutaraldehyde diffusion rate and the brownish colouration of the surface and deeper cortical regions, indicative of good glutaraldehyde fixation of 1 mm slices, was done for 2 hours. A secondary fixation in 1% osmium tetroxide-0.1 M phosphate buffer, pH 7.4, was carried out for 1–2 hours at 4°C. Black staining of the cortical slices was also observed under a stereoscopic microscope to check osmium tetroxide diffusion rate and quality of second-

ary fixation. They were then rinsed for 5–10 minutes in phosphate or cacodylate buffer of a similar composition to that used in the fixative solution, dehydrated in increasing concentrations of ethanol, and embedded in Araldite or Epon. For proper orientation during the electron microscope study and observation of cortical layers, $\sim 0.1\text{--}1\ \mu\text{m}$ thick sections were stained with toluidine blue and examined with a Zeiss photomicroscope. A light microscope study of the capillary wall, neurons, and glial cells was performed. Ultrathin sections, obtained with Porter-Blum and LKB ultramicrotomes, were stained with uranyl acetate and lead citrate and observed in a JEOL 100B transmission electron microscope (TEM) at magnifications ranging from 30 000–90 000 \times . For each case, ~ 50 electron micrographs were analysed.

Neurosurgical and microscopic study

Case 1

HRF. An 18-year-old female patient with contusion and fracture of frontal region. Biopsy sample of left frontal cortex showed severe neuronal and neuroglial oedema with remarkable enlargement of the extracellular space and presence of proteinaceous oedema fluid in the neuropil with abundant fibrinous organization. There was also increased capillary permeability with massive endothelial vacuolar transport. The myelinated axons showed an increased number of neurofilaments and microtubules, presence of necrotic membranes, myelin vacuolization, rupture of the axolemma, a moderately increased amount of oligodendroglial ad-axonal layer and formation of myelin ovoids.

Case 2

HSCR. Brain trauma. Right parieto-temporal subdural haematoma. Biopsy sample of right temporo parietal cortex. A 26-year-old male patient with skull trauma and fracture in right temporo parietal region, tonic-clonic convulsions and disorders of behaviour. This case showed severe vasogenic brain oedema with a notably increased endothelial vacuolar transport, thickening of basement membrane and breakdown of astrocytic perivascular glial end-feet layer. The perivascular neuropil showed extremely enlarged extracellular lacunar spaces, in which fragmented degenerated axons and myelin ovoids were observed. These spaces appeared electron lucent and were occupied by non-proteinaceous oedema fluid. The neurons and neuroglial cells were remarkably swollen. The myelinated axons showed advanced degeneration.

Results

Light microscopy examination of traumatically injured frontal cortex in the patient with severe frontal contusion (case 1) after 8 days of evolution time, showed at the perivascular region, marked vasogenic oedema with perivascular and intraparenchymatous haemorrhages, enlarged perivascular spaces and severe oedema (status spongiosus) of neighbouring brain neuropil (figure 1). The transmission electron microscopic examination of the capillary wall showed increased vacuolar and vesicular transendothelial transport (figure 2) and a thickened capillary basement membrane.

The light microscopic study showed dark ischaemic pyramidal and non-pyramidal neurons, oedematous satellite and interfascicular oligodendrocytes and

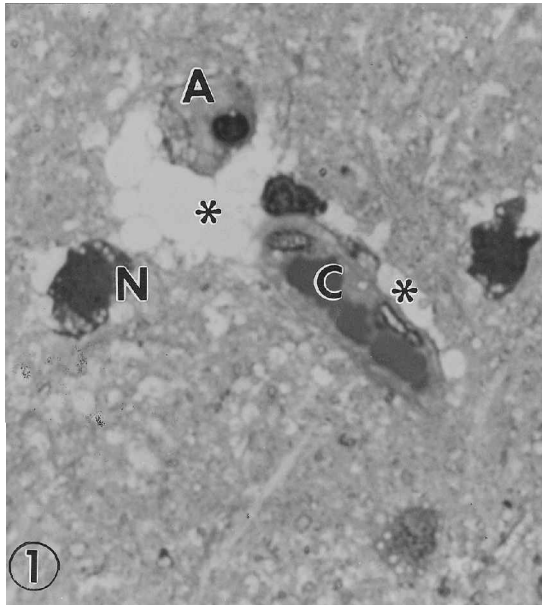


Figure 1. Case 1. Photomicrograph of frontal cortex showing a longitudinally sectioned capillary (C) surrounded by enlarged perivascular spaces (asterisks). The severe oedema of perivascular neuropil indicates the breakdown of the blood-brain barrier. Dark ischaemic non-pyramidal neurons (N) and a swollen astrocyte (A) are also seen. Magnification = $\times 375$.

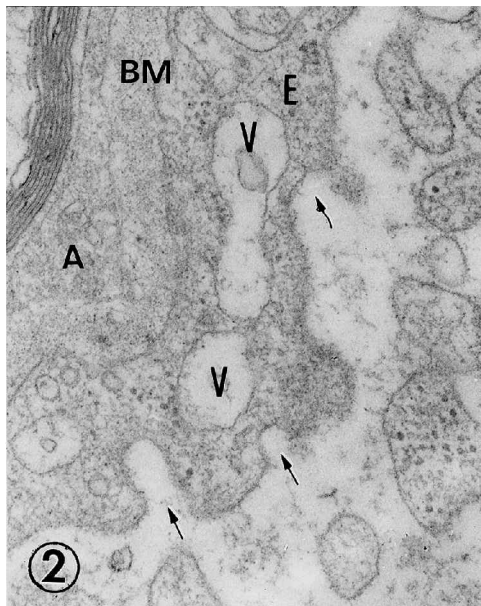


Figure 2. Case 1. Electron micrograph of capillary wall showing at the level of endothelial cell (E) the increased vacuolar (V) transendothelial transport. The basement membrane (BM) appears thickened and the perivascular astrocytic end-foot (A) is swollen. The arrows indicate the endocytic process at the level of endothelial luminal plasma membrane. Magnification = $\times 60\,000$.

remarkably swollen astrocytes (figure 3). Transmission electron microscopy showed oedematous dense neuronal cytoplasm with enlarged endoplasmic reticulum canaliculi and cisterns and swollen mitochondria (figure 4). The neighbouring neuropil exhibited clear and dark, swollen varicose dendritic profiles and numerous degenerated asymmetric axo-dendritic synapses devoid of perisynaptic glial ensheathment. The degenerated presynaptic endings showed progressive disappearance of synaptic vesicles (figure 5). In areas of severe oedema, the degenerated asymmetric axo-dendritic synapses showed notably swollen postsynaptic dendritic endings with fragmented limiting plasma membranes.

The light microscope examination of the parieto-temporal cortex in the patient with subdural haematoma (case 2), surgically re-operated after 2 years of evolution time, showed perivascular haemorrhages, status spongiosus of brain parenchyma, swollen, clear neurons, dense satellite and interfascicular oligodendrocytes, severely oedematous astrocytes and a degenerated myelinated axon (figure 6). At the light microscopy level, close examination of degenerated myelinated axons showed the axonal sprouting process (figure 7), suggesting a new reinnervation process of the injured brain parenchyma. Examination of the neuropil with the transmission electron microscope (figure 8) showed a myelinated axon at its branching axonal ramification site. The newly-formed axonal ramifications apparently established asymmetric axo-dendritic synaptic contacts either with the same swollen dendritic shaft (figure 9) or with different postsynaptic dendrites. Higher magnification of this area (figure 10) showed numerous asymmetric axo-dendritic synapses featured by a great amount of spheroidal and enlarged synaptic vesicles associated to the pre-synaptic membrane. Exocytotic vesicle sites, widened synaptic clefts in direct continuity with the enlarged extracellular space and retraction and disappearance

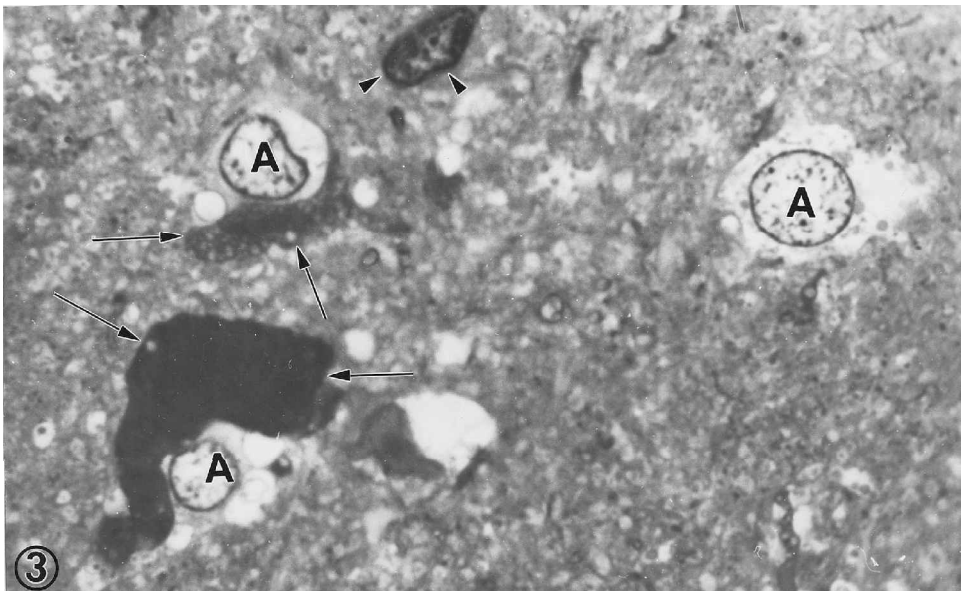


Figure 3. Case 1. Photomicrograph of frontal cortex displaying two dark ischaemic pyramidal neurons (arrows), oedematous interfascicular oligodendrocytes (arrowhead) and remarkably swollen satellite and interfascicular astrocytes (A). Magnification = $\times 520$.

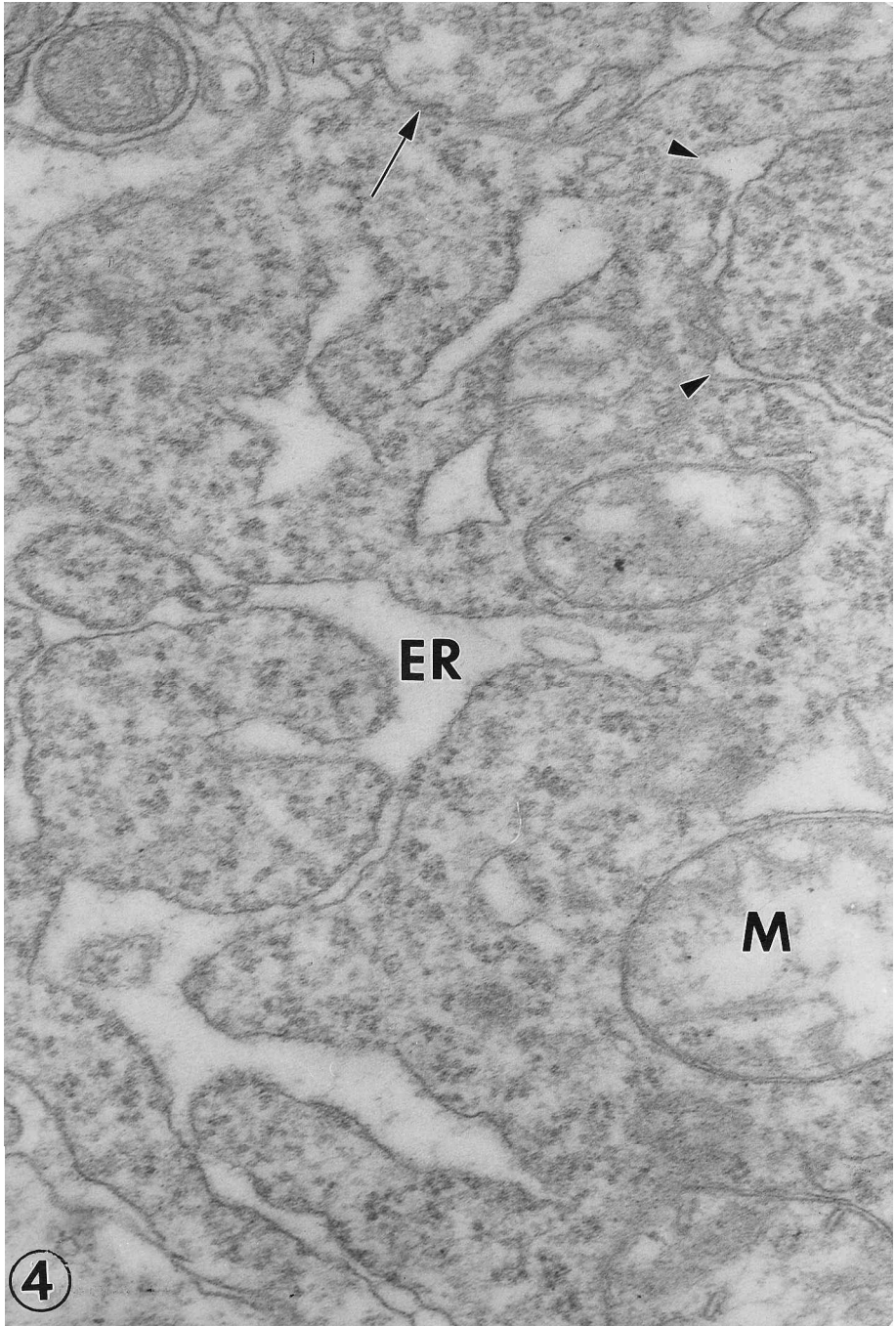


Figure 4. Case 1. Electron micrograph showing an oedematous cortical neuron, which exhibits dilated endoplasmic reticulum (ER) and perinuclear cistern (arrowheads), swollen mitochondria (M) and an axosomatic synapse (arrow). Synaptic membrane specializations are not seen. Magnification = $\times 60\ 000$.

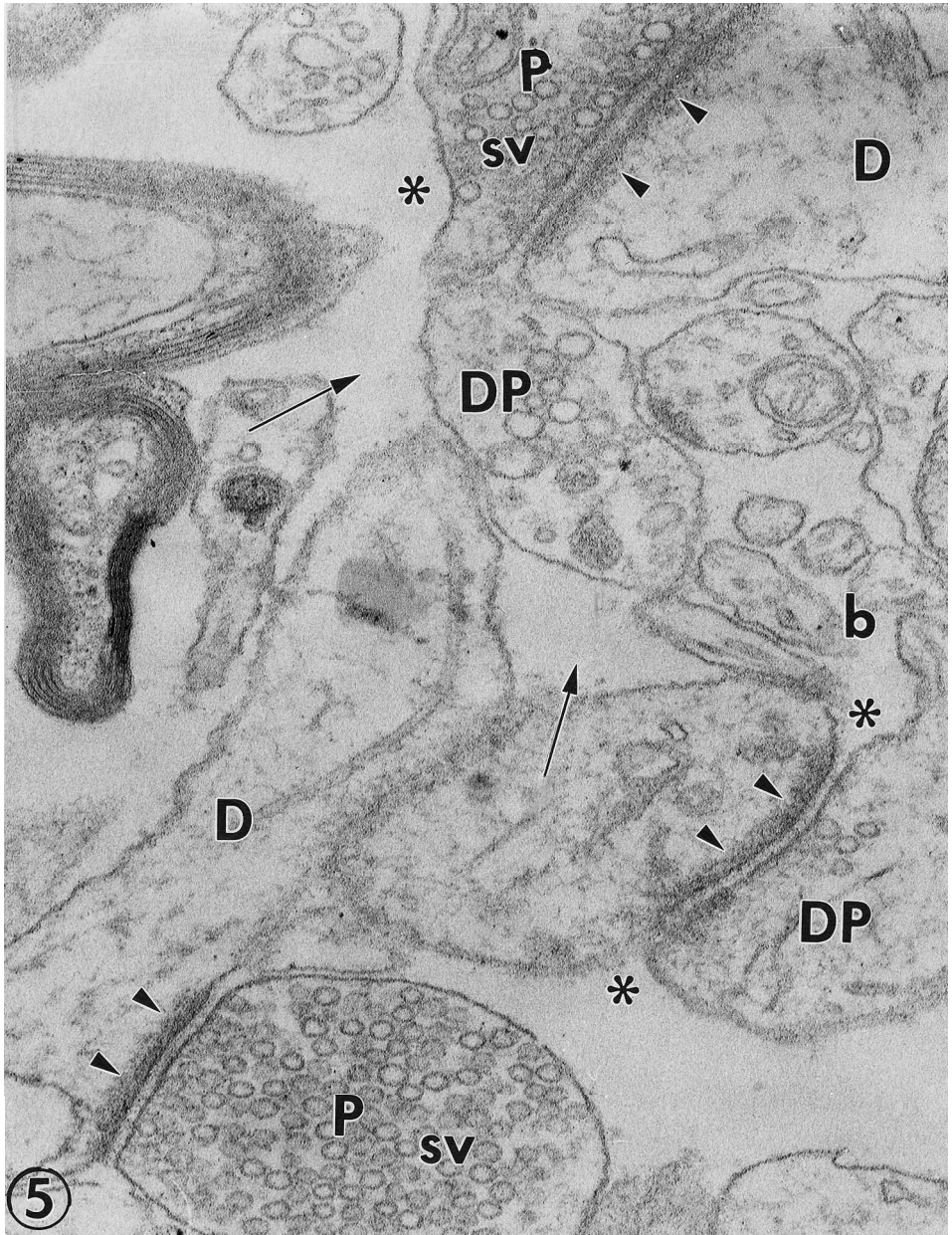


Figure 5. Case 1. Electron micrograph of frontal cortex neuropil illustrating clear degenerated dendritic processes (D), predominant swollen, asymmetric axodendritic contacts (arrowheads) and an enlarged extracellular space (arrows). The presynaptic knob (P) contains closely aggregated spheroidal synaptic vesicles (sv). The degenerated presynaptic ending (DP) showed enlargement and few synaptic vesicles. The dilated synaptic cleft (arrowheads) appears in continuity with the enlarged extracellular space, and the glial perisynaptic astrocytic cytoplasm is absent (asterisks). Magnification = $\times 60\ 000$.

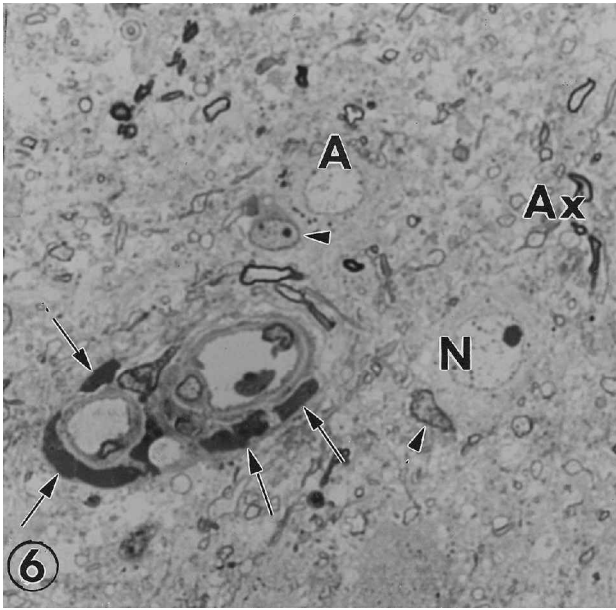


Figure 6. Case 2. Photomicrograph of right temporo-parietal cortex showing perivascular haemorrhages (arrows), status spongiosus of brain parenchyma, swollen clear neuron (N), dense satellite and interfascicular oligodendrocytes (arrowheads), oedematous astrocyte (A) and degenerated myelinated axons (Ax). Magnification = $\times 375$.

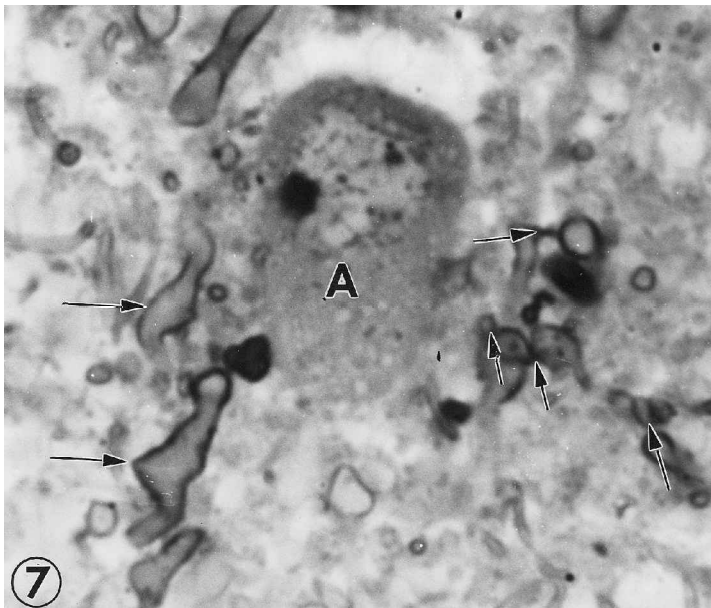


Figure 7. Case 2. Photomicrograph of a severely oedematous neuropil showing a swollen astrocyte (A) surrounded by degenerated myelinated axons (long arrows). The short arrows show the sprouting myelinated axons at their branching sites. Magnification = $\times 375$.

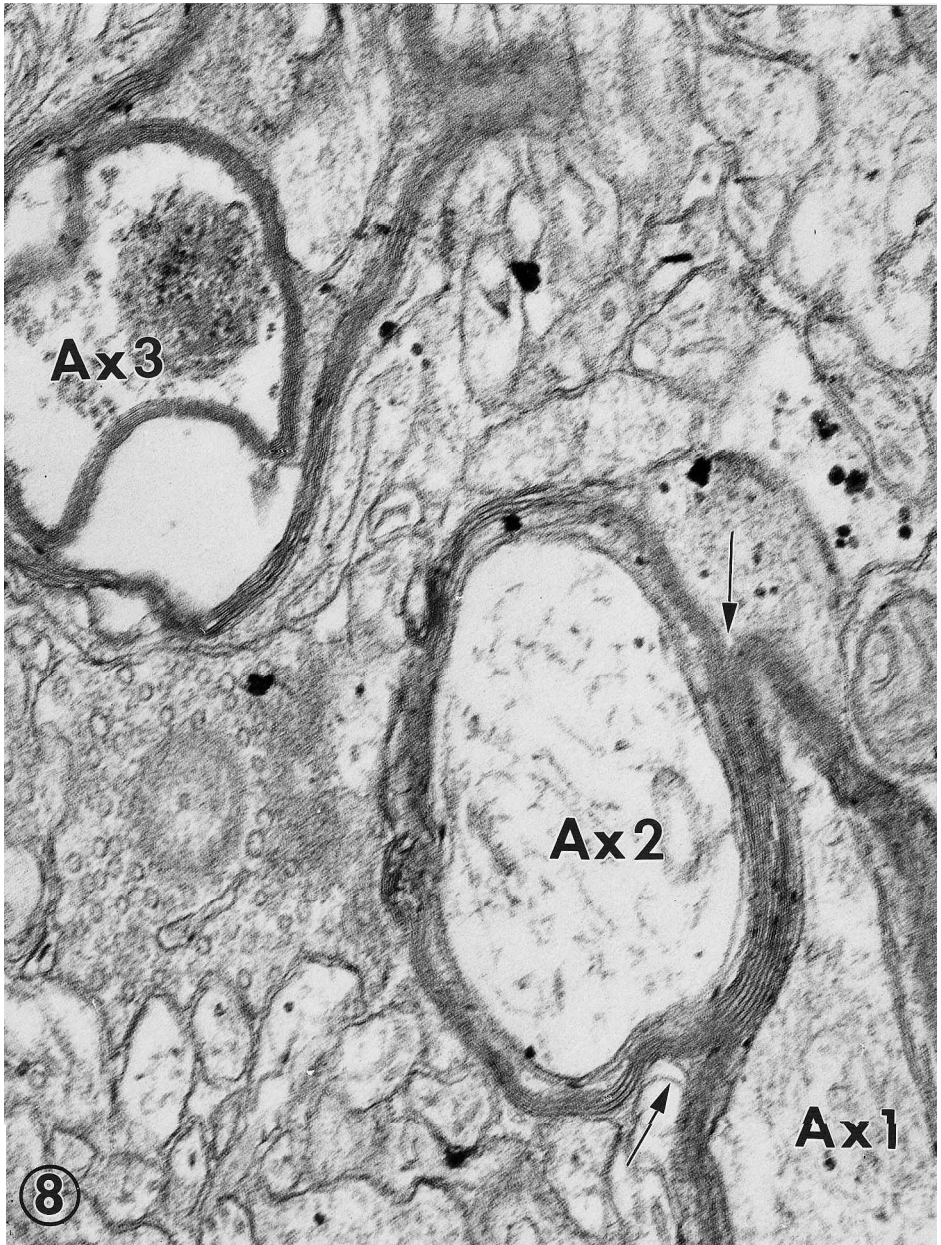


Figure 8. Case 2. Right parietal cortex. Right parieto-temporal subdural haematoma. Two years of evolution time. Electron micrograph of moderately oedematous neuropil showing two neighbouring myelinated axons (Ax1, Ax2) at their branching site (arrows). Another degenerated myelinated axon (Ax3) is also observed. $\times 60.00$.

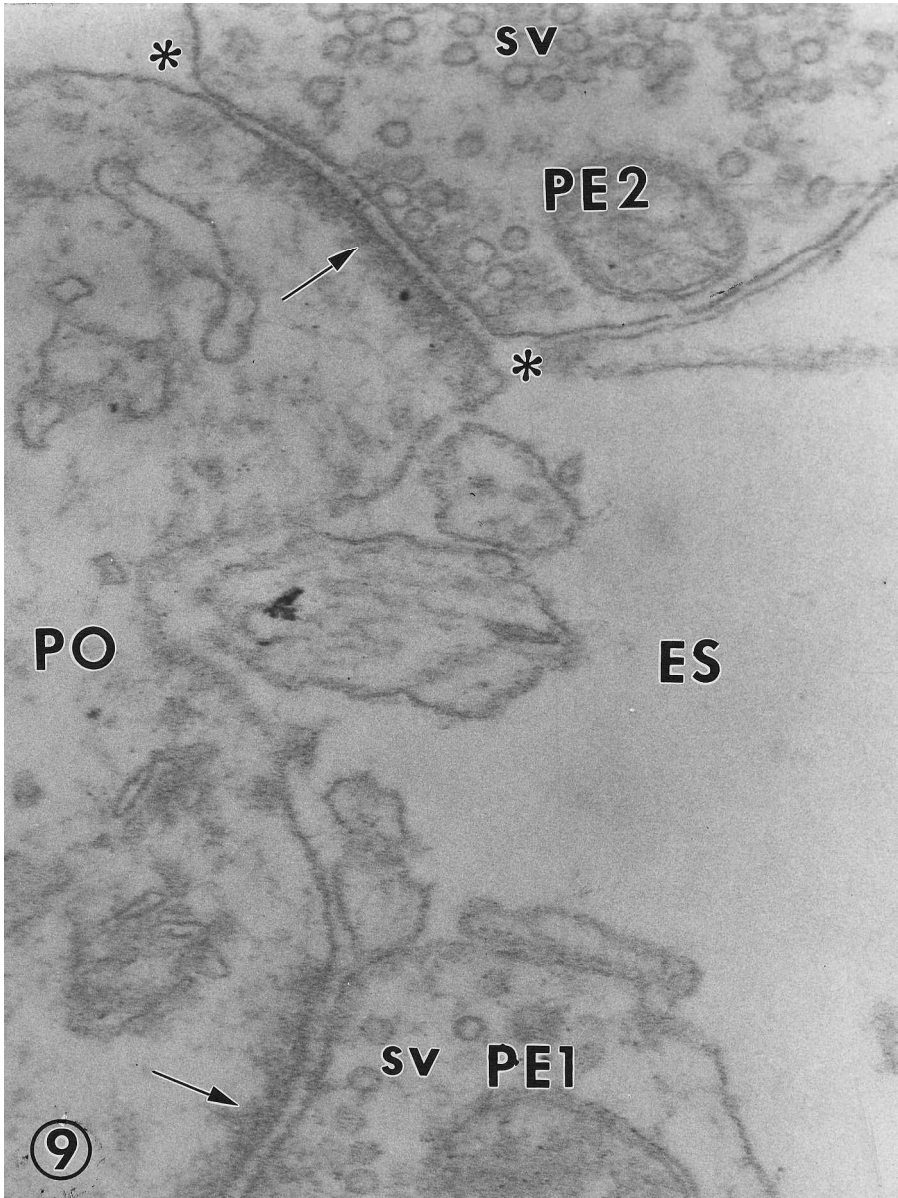


Figure 9. Case 2. Right parietal cortex. Right parieto-temporal subdural haematoma. Two years of evolution time. The presynaptic endings (PE1 and PE2), containing spheroidal synaptic vesicles (sv) appear making asymmetric synaptic contacts (arrows) with the same swollen postsynaptic dendrite (PO). The perisynaptic glial sheath is absent (asterisks). Note the lacunar enlargement of extracellular space (ES), containing non-proteinaceous oedema fluid. Magnification = $\times 90\ 000$.

of perisynaptic glial layer were also seen. In most axodendritic synapses, a remarkably swollen postsynaptic dendrite was observed (figure 11). Some axo-dendritic synapses showed, in severely oedematous neuropil, a process of synaptic disassembly with aggregated synaptic vesicles toward the presynaptic membrane density, net separation of pre- and postsynaptic structures and disappearance of pre- and postsynaptic membrane complex (figure 12).

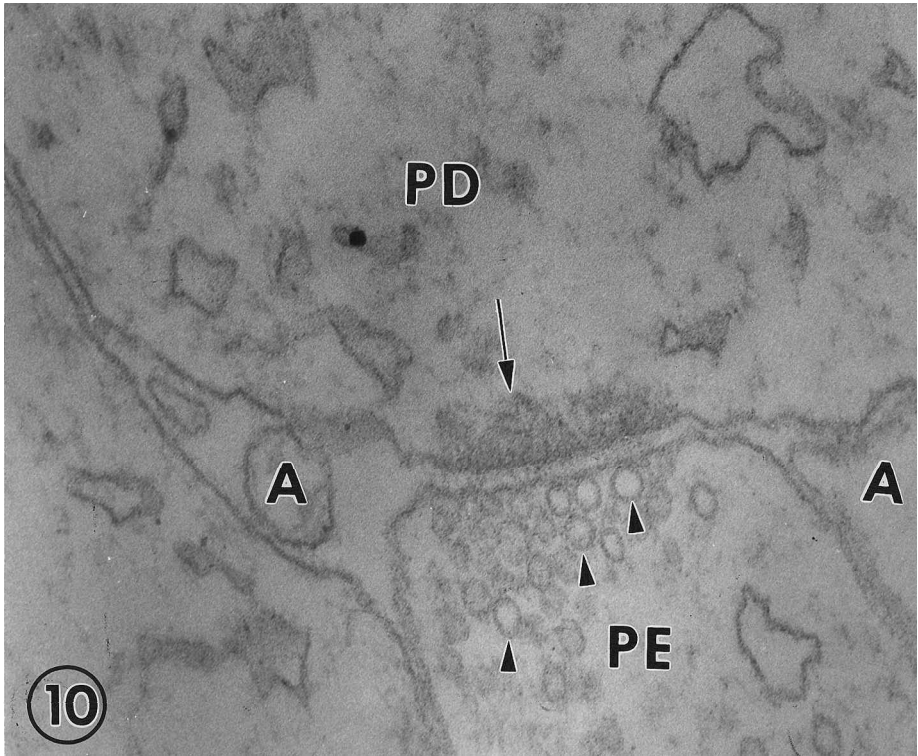


Figure 10. Case 2. Axodendritic asymmetric synapse showing at the presynaptic ending (PE) aggregated synaptic vesicles toward the presynaptic membrane (arrowheads). Most of them appear to be discharging into the dilated synaptic cleft. Note the net separation of the perisynaptic astrocytic (A) cytoplasm from the synaptic active zone and the prominent postsynaptic density (arrow) at the swollen postsynaptic dendrite (PD). Magnification = $\times 90\,000$.

Discussion

The present study has reported the alterations of oedematous human cerebral cortex in two patients with brain trauma and post-traumatic epilepsy. Both cerebral cortices exhibited perivascular and parenchymatous haemorrhages, ischaemic dark neurons, dense oligodendrocytes, swollen astrocytes, degenerated and sprouted myelinated axons, abundant degenerated axo-dendritic symmetrical synapses and synaptic disassembly.

Light and electron microscopic observations revealed a blood-brain barrier breakdown. These structural findings observed after traumatic brain injury (TBI) are apparently responsible for the enhanced neuronal activity, seizures and convulsive crisis clinically observed in the patients under study. The ischaemic damage of neurons and capillaries as reported in the present paper is similar to that reported by Lu [2] in cryptic temporal lobe epilepsy.

At the light microscopic level, one found sprouting of myelinated axons in the parieto-temporal cortex of a patient surgically reintervened after 2 years of evolution of the TBI, which is probably associated with new innervation processes and formation of multiple ectopic synapses. According to Salin *et al.* [11], a significant degree of axonal reorganization takes place in the chronically injured cortex. This

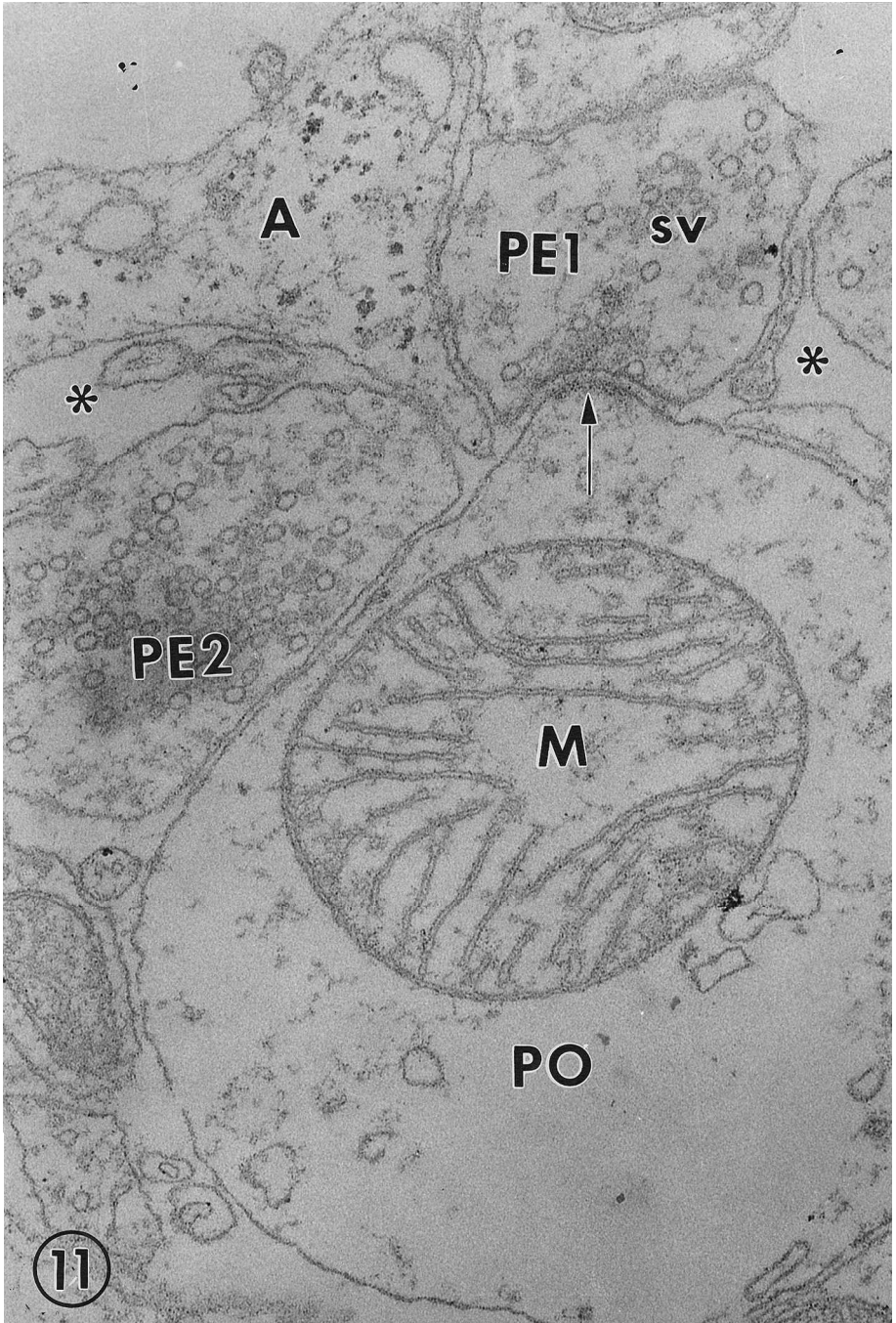


Figure 11. Case 2. An asymmetric axo-dendritic synapse (arrow) appears showing a distorted presynaptic ending (PE1) with scarce isolated synaptic vesicles (sv). The postsynaptic ending (PO) appears remarkably swollen and contains a huge mitochondria (M), another swollen presynaptic ending (PE2) and the dilated extracellular space (asterisks) are also observed. Note the net separation of perisynaptic astrocytic (A) cytoplasm containing β type glycogen granules (arrowheads). Magnification = $\times 60\,000$.

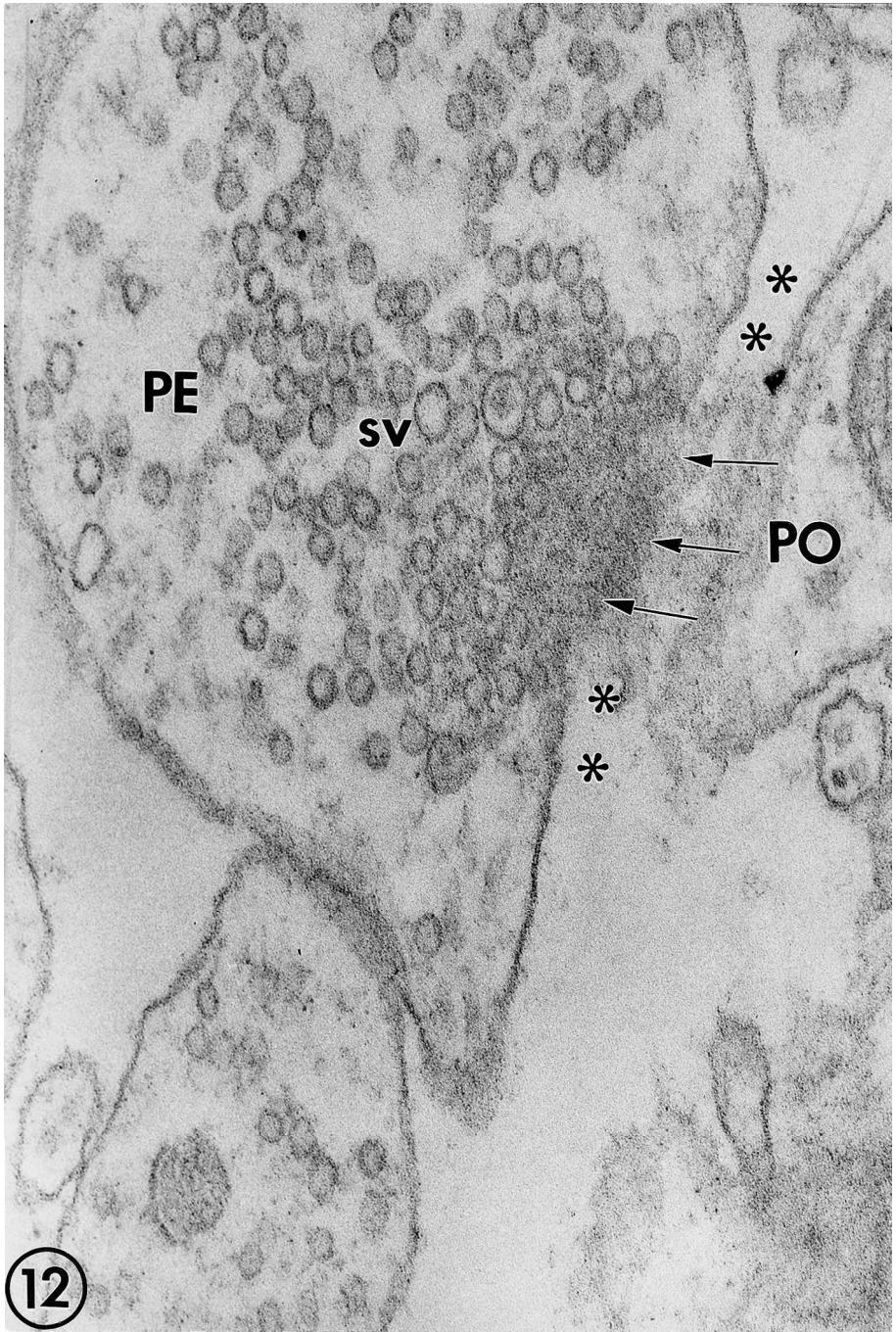


Figure 12. Case 2. A process of synaptic disassembly induces separation of presynaptic ending (PE) from postsynaptic ending (PO). The presynaptic ending appears swollen and shows an irregular pattern of synaptic vesicle (sv) distribution. Note the coalescence of synaptic vesicles toward the presynaptic density (arrows). Pleomorphic synaptic vesicles are seen. The synaptic cleft appears dilated and in continuity with the enlarged extracellular space (asterisks). The presynaptic glial ensheathment is lacking. Magnification = $\times 90\,000$.

finding might be due to an adaptive mechanism for recovery of function after injury, or could be a maladaptive process, which could play an important role in the generation of epileptiform events by increasing the number and density of synaptic contacts between neurons. It is possible to assume, as illustrated in figures 6 and 10, that the presence of numerous asymmetric axodendritic contacts, which have been correlated with excitatory synapses [19], induces synaptic reorganization and the formation of recurrent excitatory circuits. This reorganization could be responsible by an imbalance between excitatory and inhibitory circuits in the patients with post-traumatic seizures. Formation of recurrent excitatory circuits and inhibitory circuits have also been reported in the dentate gyrus of epileptic rats [20]. In addition, sprouting of mossy fibres and multiple ectopic asymmetric synapses have also been signalled in the hippocampal formation after application of high K^+ or excitatory amino acids [6].

The present paper has described some synaptic changes of axo-dendritic asymmetrical synapses, such as coalescence of spheroidal synaptic vesicles, aggregation of synaptic vesicles toward the presynaptic membrane and numerous exocytotic vesicle-sites. These changes, observed at the level of presynaptic endings, have been interpreted as signs of synaptic degeneration [21]. Other submicroscopic changes, such as the absence of perisynaptic glial ensheathment, enlargement of the synaptic cleft and the extracellular spaces are mainly related with vasogenic brain oedema and ischaemia [22].

Dark, swollen pyramidal and non-pyramidal neurons, as reported in the present study, were also described by Ribak and Baram [12] in several limbic structures, mainly hippocampal CA3 pyramidal cells, after CRH-induced status epilepticus in infant rats. The shrunken appearance and selective loss of these neurons were interpreted by the authors as incompatible with necrosis as the mechanism of degeneration. Loss of inhibitory synapses on the soma and axon initial segment of pyramidal cells in human epileptic peritumoural neocortex, and their implications for epilepsy, have been reported by Marco *et al.* [23]. Degeneration of neurons and reactive gliosis have previously been reported in TBI [24, 25]. Similar findings have been reported by Ribak *et al.* [26] in the temporal lobe of rhesus monkeys with partial seizures after alumina gel injections, as also observed in human temporal lobe epilepsy.

Little neuronal argyrophilia with some features of apoptosis was reported by Friedman [14] after kainic acid induced-status epilepticus. Kainate-induced apoptotic cell death in hippocampal neurons was also found by Pollard *et al.* [10]. Neuronal necrotic and apoptotic death have been reported in the lithium-pilocarpine model of status epilepticus [15] in rats during development. In this study, signs of neuronal apoptotic death, such as nuclear chromatin condensation and marginalization, were not observed.

There are now converging lines of evidences from several animal studies to suggest that excitotoxic damage to neurons and glial cells may be developed as a consequence of excessive release of excitatory amino acids after primary impact injury, ischaemic events and haematoma [27]. Figures 6 and 10–12 illustrate images of numerous exocytotic vesicles sites at the presynaptic endings, which suggest increased neurotransmitter release. Glutamate released by the traumatic injured brain [1, 27], may reach toxic levels at the enlarged synaptic cleft to cause seizures and neuronal and glial cell damage. In addition, as illustrated in figures 6, 11 and 12, the glial perisynaptic layer is absent, suggesting that conversion of glutamate to

glutamine by the perisynaptic astrocytic cytoplasm would not take place; which could additionally increase glutamate levels at the synaptic cleft and explain the hyperexcitability of nerve cells and the induction of seizures in the patients studied. It is important to mention that, in the patients with seizures, the four major mechanisms causing brain damage after human head injury (contusion, haematoma, hypoxia and diffuse axonal injury [27]) were present.

Some considerations on clinical application and epilepsy research

In a patient with TBI, it is important to measure by high pressure liquid chromatography (HPLC) the level of amino acids in cerebrospinal fluid and plasma to study the levels of excitatory amino acids, to have an idea about the presumed relation between the levels of excitatory amino acids and epileptogenesis [28]. Also, to study the degree of excitotoxicity in the epileptic patient, analysing the plasma levels of glutamate. In addition, it is very important to study, by means of positron emission tomography of frontal, parietal and temporal cortex, the presence of hyperexcitable neuronal circuits. From the neuropathological point of view, it is recommended to study, by light and electron microscopy, the process of nerve cell apoptosis in epileptic patients.

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