Chapter VIII

HISTOMORPHOLOGICAL CHANGES IN RAT CEREBRAL CORTEX FOLLOWING EXPERIMENTAL EPILEPTOGENESIS

Introduction

At various times in history, epilepsy has been thought to be an unidentified supernatural force, at times forms of evil spirits, devils, demons or black magic. Again many regarded epilepsy nothing but a form of mental illness.

Even with time when a scientific idea started to take shape regarding the syndrome; researchers accepted 'it' to be a disorder only of function but not of structure (Townsend, 1976). However, autopsy studies of epileptic patients did reveal lesions of the gray matter of the brain. Loss of neurons and proliferation of macrophages, astrocytes and vessels were observed both at the putamen and parieto-occipital cortex (Oldfors et al, 1987).

Such contradictory references prompted us to study the structural changes in the brain, if any, caused due to epileptogenesis.

Materials and Methods

After the preparation of epileptic rat models the rats were anaesthetized with pentobarbital and stained with the brain of the anaesthetized animal was perfused through intra cardiac route with normal saline and followed by 10% formalin. Cerebrum was then dissected out in cold, processed routinely for serial paraffin sections. The sections were stained with Hematoxylin-Eosin, Cresyl Violet and Bielchowsky’s silver stain and studied subsequently microscopically. Cell counts were made with the help of camera lucida (Deb et al, 1964).
Results

Histological picture of acute PCN epileptic focus

In acute PCN epileptic animals there was very little change in the histological picture of the cerebral cortex as compared to the mock operated saline treated animals (Plate 20 (A1)). Some microvacuolations (Plate 20 (A2)) and zone of coagulation with necrosis at the pial surface, denoted by close adhesion and some oedematous change at the deeper layers particularly the ganglionic and pyramidal cell layers were observed. In those brains where single dose of PCN was applied the involved cortical area showed a lesion semicircular in shape with its base towards the surface and apex towards the deeper parts up to the white matter.

Histological appearance in chronic PCN epileptic focus

In all the animals, lesions provoked by repeated topical PCN application were plainly evident at the autopsy. Histologically they were quite different from those produced by saline injection in mock operated animals. A semicircular or often cylindrical lesion of approximately 1-2 mm diameter and 3-4 mm deep were seen microscopically. Sometimes the epileptogenic lesions were very widespread with multiple dissolute appearance of the piamater. The lesions had a crater-like appearance with raised border adhering to the duramater. Serial histological sections revealed the localized lesions. These features were distinctly different from those of mock operated saline treated animals. In the most superficial layer (molecular layer) there was significant decrease in the nerve cell while in the deeper layers and at the periphery the cells were shrunken. More severe reactions around the chronic epileptic focus were found in those animals where PCN was injected repeatedly and the animals were sacrificed after 30 days (Plate 20 (A3)). In these cases the epileptogenic lesion was comparatively much wider showing a central necrotic area extending from the surface to
Plate 20: Photomicrographs of Haematoxylin-Eosin stained 10μm coronal sections from the cerebral cortex of rats after single (A) and repeated (thrice) penicillin application (A) in comparison to saline injected control animal (A). Photomicrographs illustrate examples of higher number of cells and lower number of vacuoles in the pyramidal cell layer in the control section as compared to the increased vacuolization and lowered number of cells in the outer pyramidal cell layer of epileptic rat cortex. Changes in staining quality of damaged neurons [dark-stained neurons, with shrunken cytoplasm, surrounded by perineuronal vacuoles] (A) are clearly visible compared to normal appearing round cells (A).
the deeper layer with perifocal glial proliferation and increased vacuolization. The average nucleus size was found to decrease by 30% while a decrement of 56% was observed in the average cell size. In the deeper layer there was massive infiltration of small round cells. In the large pyramidal cell layer, the cell showed a decrease in size by 25% to that of the controls. Sometimes an inflammatory reaction was seen from the peripheral part to the central area and some distinct zones of coagulation necrosis, edematous zone with a significant loss of the ganglionic cells and zone of round cell infiltration could be seen. In the epileptiform sections stained with Cresyl Violet it could be seen that the Nissl granules as well as the pyramidal cells were dispersed and their numbers were also lessened compared to the control and mock operated ones (Plate 21). Clumping of neural cells and degenerated nerve fibres were observed in the cortical sections of epileptiform animals upon staining with Bielchowsky’s silver stain. A higher magnification of the pictures of the experimental sections stained with the same Bielchowsky’s silver stain showed the eccentric location of the nuclei and complete breakdown and disappearance of the axons, disintegration and decrease in the number of nerve cells as compared to the control ones (Plate 22).

**Histological appearance in PTZ epileptics**

In the PTZ epileptic models not much cytological destruction was observed though the average cell and nuclear size had decreased to some extent. The observed neuronal alterations reflect the temporal evolution of most neurons after injury. Other observed morphological alterations were dark blue - stained cells with triangular shape, microvacuolation or shrinkage of the cell body (Plate 23). Neurons with altered morphology and signs of degeneration were present in the whole cortex. However the aberrations were reversible in nature and were not taken into account in the
Plate 21: Photomicrographs of Cresyl violet stained 10μm coronal sections from the cerebral cortex of rats after single and repeated (thrice) penicillin application (B₂ & B₃ respectively) in comparison to control animal (B₁). Photomicrographs illustrate examples of pyramidal cells and normal nissl granules in the control sections as compared to dispersed and disintegrated pyramidal cells and nissl substances in epileptic rat cortex.
Plate 22: Photomicrographs of Bielchowsky's silver stained 10μm coronal sections from the cerebral cortex of rats after single and repeated (thrice) penicillin application (C₂ & C₃ respectively) in comparison to sham control animal (C₃). Photomicrographs illustrate examples of normal axons and pyramidal cells in the control section as compared to the degenerated axons and lowering in the number of pyramidal cells in C₂ and clumping of neural cells in C₃.
Plate 23: Photomicrographs of Haematoxylin-Eosin stained 10μm coronal sections from the cerebral cortex of rats after pentylenetetrazol application (D₁ & D₂). Photomicrographs illustrate microvacuolations and shrinkage of cell body.
counting method. Unlike the chronic PCN model PTZ failed to make any permanent impression on the brain cytology.

**Histological appearance in amygdaloid kindled epileptic rats**

Amygdaloid kindling did result in structural damage of the rat brains. The kindled brains were histologically quite different from mock operated animals. Brains taken from amygdaloid kindled rats showed atrophy, visible by general inspection. Serial histological sections revealed the localized lesions. The number of neurons was reduced in all the areas of the brain as compared with the densities in the controls. The neuron loss was most marked (upto 50%) in the granule cells. The average nuclear size was decreased by 25% while the average cell size decreased by 44%. Neuronal density progressively declined as seizure frequency increased. The kindled focal area was damaged most (Plate 24, 25). The focus and adjacent areas were marked with swelling and vacuolation of cell body (Plate 26), rupture, dissociation and dissolution of nucleus, cell lysis with inflammation injuring adjacent tissues. Shrunken, angulated and clumped neurons were abundant within the zone adjacent to the stimulation site (Plate 27).

**Discussion**

The present data document dynamic changes in the pattern of neuronal damage. It has been demonstrated here that epilepsy induced neuronal damage occur during the chronic period chiefly. Though limited neurodegeneration is observed during the acute phase. The data is in corroboration with the findings of K

ová et al (2002) who found degeneration of mediodorsal nucleus (MD) of the thalamus to be mainly related to age at status epilepticus (SE) onset and the chronicity period (3 months) after SE. This SE induced damage in the MD thalamic nucleus is attributed to glutamate-induced
neurotoxicity. The role of excessive glutamatergic excitation in development of SE-induced neuronal damage has also been described by Turski et al (1989).

The results of the present study proves the old adage that epilepsy is only a disorder of function and not of structure (Townsend, 1976) a myth.
Plate 24: Photomicrograph (E,) of Haematoxylin-Eosin stained 10µm coronal sections from the cerebral cortex of amygdaloid kindled rat. Stimulation was given in the basolateral nucleus of amygdala for 14 days and the rat was sacrificed after 15 days. Photomicrograph illustrate intense vacuolization and damaged neuron.

Plate 25: Photomicrograph (F,) of Cresyl violet stained 10µm coronal sections from the cerebral cortex of amygdaloid kindled rat. Stimulation was given in the basolateral nucleus of amygdala for 14 days and the rat was sacrificed after 15 days. Photomicrograph illustrates damaged kindled focal area.
Plate 26: Photomicrographs of Bielchowsky’s silver stained 10μm coronal sections from the cerebral cortex of sham control (G₁) and amygdaloid kindled rat (G₂). Stimulation was given in the basolateral nucleus of amygdala for 14 days and the rat was sacrificed after 15 days. Photomicrographs illustrate intense vacuolization and clumping of neural cells.
Plate 27: Photomicrographs of Bielchowsky's silver stained 10μm coronal sections from the cerebral cortex of amygdaloid kindled rat. Stimulation was given in the basolateral nucleus of amygdala for 14 days and the rat was sacrificed after 30 days. Photomicrographs (H₁ & H₂) illustrate shrunken, angulated and clumped neurons in the zone adjacent to the stimulation site.