CHAPTER IV

BEHAVIOURAL AND ELECTROENCEPHALOGRAPHIC
CHANGES IN EXPERIMENTAL EPILEPTIC RATS

Introduction

Any study of epileptogenesis is incomplete without a thorough knowledge of neuropsychological and behavioral responses of the experimental subjects. Indeed, without a complete, true behavioral picture primary diagnosis of epilepsy is impossible. Also in cases of chronic epilepsy regular notation of behavior changes is necessary to keep track of the status of the disease.

Epilepsy is most easily defined in physiological terms, being 'the name for occasional, sudden, excessive, rapid and local discharges of grey matter'. But half a century had to elapse before it became possible to record such discharges means of electroencephalogram or EEG. A lot of innovation has taken place since the inception of EEG. But till today EEG remains to be the primary tool for epilepsy diagnosis and characterization.

So keeping in view of developing experimental models of epilepsy, it seemed pertinent to study the behavioral changes and electroencephalographic recordings in rats following penicillin, pentylenetetrazol and amygdaloid kindling treatments. This chapter focuses mainly on the motor behavior abnormalities and associated EEG changes of the chronic and acute epileptic rats.

Materials and Method

As mentioned in the previous chapter (Methodology and Techniques) male rats (laboratory bred Holtzman strain) weighing between 150-200g were used for this study. All the animals were maintained with standard diet and drinking water ad libitum.
rats were also checked for any pre-existing epilepsy. Also their general health, food and drinking habits, micturation and fecal output were noted. For the preparation of PCN induced epileptic rats 100 IU sodium benzyl penicillin G solution was stereotaxically injected in the somatosensory cortex of the rats’ brain. For the PTZ model PTZ (20 mg/kg) was injected intraperitonially. Amygdaloid kindled rats were prepared by stimulation of the basolateral nucleus of amygdala. The behaviour pattern of each rat was analyzed as has been described in detail in the previous chapter. Electroencephalographic recordings were taken from the surface cortex, somatosensory cortex and the amygdala.

Results

Behavioural manifestations of PCN induced epileptic rats:

In acute animals

During the observation period ranging from 6-8 hours several changes in the behaviour pattern were noted. The different stages of behavioural manifestations are represented in Fig. 4.1. Within 5-15 minutes after the application of first dose of penicillin and as soon as the effect of anaesthesia went off the animals became hypersensitive and started making circling movements in contralateral direction to which drug was applied (Plate 3). At first the head was held in neutral position without tremor. Gradually, occasional or regular twitching of the ears of the opposite side to which penicillin was applied was seen. Twitching of the facial muscles and limbs of the opposite side of drug application followed this and there was practically no resistance to passive manipulation of any of the limbs by pinpricking. The clonic movements of the contra lateral limbs (fore and hind limbs) started within 2-5 minutes, which was then followed by convulsions of the hemi body lasting for few seconds only. After several recurrences of these sequences these clonic movements and tonic convulsions moved to
the opposite side as a 'Jacksonian March' from the limbs to the entire body as a whole. These tonic and clonic flexion type of generalized convulsions actually started as a sequential event starting from the nose area— to the opposite side limbs (stage 2, 3, 4) and terminating to generalized convulsion (stage 5, Plate 4) at certain intervals and then followed by post-ictal depression and subsidence of all seizure phenomenon. During this stage the animals always showed prominent erection of tail (Plate 5). The ictal phenomenon at the early phases lasted for 20-30 seconds but later on was prolonged to minutes while the inter-seizure period was gradually lessened from 30 minutes to 2-5 minutes. During the post-ictal phase the pupil was contracted and there was frothing of the mouth along with salivation (Plate 6).

During the convulsive phase the animal lost its righting reflexes and often showed jerky or jumpy movements, throwing of the body in either direction were noticed. The sequence of such convulsive phenomenon did not persist for a long time and rarely recurred beyond 3-4 hours after penicillin application unless the same was given at higher concentration (more than 100 IU) or repeatedly. The clonic and tonic type of convolution gradually subsided and only in some instances the distal musculature jerked whereas in others the proximal muscles were involved resulting in rhythmic lifting of the limbs.

After the subsidence of recurrent convulsive phenomenon within 3-4 hours after penicillin application while the animals were thought to be quite normal, active jerky movements (clonic) could easily be provoked by hand clapping which was rarely seen in mock operated control animals. After 24 hours following single penicillin application there was no spontaneous convulsions but repeated auditory clapping or photic stimulation could induce it.

During convulsions excessive micturation and defecation was observed in the animals. However such behavioural phenomena were absent during seizure free periods.
Plate 3: Photograph of behavioural manifestation of PCN induced acute epileptic rat. 10 minutes after PCN administration in the right somatosensory cortex the animal had started moving in the anticlockwise direction.

Plate 4: Photograph of behavioural manifestation (typical stage 5 rearing posture) of PCN induced epileptic rat during acute generalized convulsion. Excessive defeacation is to be noted.

Plate 5: Photograph of PCN induced acute epileptic rat. Prominent erection of tail can be noted.

Plate 6: Photograph of PCN induced acute epileptic rat. Frothing of mouth due to hypersalivation is evidenced.
In chronic animals

The gross spontaneous epileptoid convulsive movements as seen in the acute animals were rarely seen in those animals for a prolonged period of time except immediately after repeated application of penicillin. Moreover the severity of convulsive phenomenon as perceived during the acute phase gradually decreased in the chronic preparation. In all the animals, when penicillin was applied for second or third time in the same foci, there was definite recurrence of convulsive movements of the same nature as that of the acute but to a lesser degree. The intensity and duration of such epileptoid phenomenon was much lower and gradually subsided within the next 6-8 hours. However the animals showed recurrent convulsive movements, clonic and tonic in nature, when provoked by mechanical, photic (Plate 7) or auditory stimulation. While the recurrence of spontaneous tonic and clonic seizures subsided within 3-7 days in cases of single application, it persisted for 3-4 weeks in rats after repeated application (Plate 8).

A decrease in the daily food intake was observed in the chronic epileptic animals. The anorectic nature of epileptic seizures explains the decrease in the daily food intake of the rat without any consequent change in body weight. However this factor might not be of much significance to the overall well-being and recovery of the epileptic animals.
Plate 7: Photograph of PCN induced chronic (15 days) epileptic rat. Convulsion could be triggered by external photic stimulation.

Plate 8: Photograph of PCN induced chronic (30 days) epileptic rat. The animal was in a stage of hyperexcitation as is evidence from its posture and limb extension.
Fig. 4.1: Behavioural pattern modification following penicillin induced seizures in rats. Each value represents the Mean ± SEM of eight rats. The level of seizure activity was evaluated using the modified scale of Patel et al (1988). Scores: 0= No response, 1= Gustatory movements and/or fictive scratching, 2= Tremor, 3= Head bobbing, 4= Forelimb clonus, 5= Rearing, falling and clonus.

PCN= Penicillin

**Behavioural manifestations of PTZ induced epileptic rats:**

All rats given PTZ (20 mg/kg) developed recurrent seizures. Several behavioural changes were noted following PTZ induction, however the behavioural manifestations were distinctly diverse from PCN rats. The behavioural manifestation stages are represented graphically in Fig 4.2. Unlike PCN induced epileptogenesis where the seizure activity started from the nose area in case of PTZ induced epileptogenesis seizures started with clonus of forelimb and head muscles with preserved righting ability. After PTZ application the rats showed sniffing, grooming, rearing and also climbing activities. Exploratory locomotion tended to be increased and the duration of freezing tended to decrease indicating anxiolytic behavioural changes. PTZ produced a significant suppression in social interaction behavior. The rats showed diminished offensive behaviour, exhibited reduction in competitiveness and aggressiveness, similar to the symptoms observed in human depression (Weiss et al, 1994). Within 10 minutes
of PTZ administration seizure discharges were observed. Facial movements, ear
twitching and tail raising were observed (Plate 9). There was occasional myoclonic jerks
and paroxysmal clonic convulsions accompanied by rearing and falling down. The tonic
phase was of shorter duration as compared to PCN epileptogenesis and the majority of
animals exhibited clonic seizures with rearing and loss of body control.(Plate 10, 11).
The rats never developed a mean seizure stage significantly higher than 3 after a single
PTZ administration. Convulsions were followed by significant increase in the tail-flick
latencies, at least for 30 min of the post-ictal period. The intensity and duration of PTZ
epileptic manifestation was much milder than PCN epileptics and subsided within the
next 4-5 hours. However the animals showed recurrent convulsive movements, clonic
and tonic in nature, when provoked by mechanical, photic, auditory stimulation. While
the recurrence of spontaneous tonic and clonic seizures subsided within 24 hours.

Fig. 4.2: Behvioural pattern modification following pentylenetetrazol induced seizures in rats.
Each value represents the Mean ± SEM of eight rats. Convulsive behaviour was observed for 30
min and classified in stages as described by Smialowsky (1980): Scores: 0= No behavioural
changes, 1= Facial movements, ear twitching and tail raising, 2= Myoclonic jerks of the whole
body, 3= Clonic convulsion with rearing, 4= Clonic convulsion with falling down and loss of
body control.
PTZ= Pentylenetetrazol
Plate 9: Photograph of PTZ induced epileptic rat. Raised tail and hind limb extension can be noted— atypical of stage 1 and 2 seizure phase.

Plate 10: Photograph of PTZ induced epileptic rat (typical stage 3 rearing posture) during convulsion. Excessive defeacation can be noted.

Plate 11: Photograph of PTZ induced epileptic rat. Typical stage 4 posture-convulsion with falling down and loss of body control.
**Behavioural manifestations of amygdaloid kindled rats:**

Behavioural manifestations following amygdaloid stimulation were not much initially when compared to PCN and PTZ induced behavioural changes. There was no initial visible seizure but within 30-50 seconds after each stimulation the rats showed excessive eye and whisker twitching along with continuous head nodding, facial scratching (Plate 12), erection and rotation of tail and occasional lipsmacking. From stage 3-4 kindling onwards there was repetitive limb clonus (Plate 13), rearing and falling. Fully kindled rats displayed generalized tonic-clonic convulsions with rearing (Plate 14) and falling. There were five types of specific behavioral seizure correlates, two associated with focal AD and three associated with generalized AD. Focal AD was associated with staring and eye-twitching, which are equivalent to stage 1 and stage 2 kindled seizures, respectively. Generalized AD was accompanied by additional behavioral seizure correlates, including salivation often associated with lip-sacking (also called chewing) followed by repetitive limb clonus before or after head clonus (also called head nodding) - events equivalent to a stage 3 kindled seizure. The different stages of behavioural manifestations are represented graphically in Fig 4.3.

**Electroencephalographic changes in PCN induced epileptic rats:**

In our colony of control rats we did not come across any case of spontaneous spike and wave discharge as are known to occur in genetically epileptic or epilepsy prone rats. Therefore the epileptic activity seen in our PCN and PTZ -induced epileptic rats, amygdaloid kindled rats was solely due to experimental induction. The electrical activity in the brain of the normal mock operated unrestrained rats was predominantly rapid with varying voltages (50-200μV), but no high voltage spike activity could be seen in these animals either spontaneously or when provoked by flickering light. The normal EEG pattern showed predominance of low voltage fast waves or β waves with few high
Plate 12: Photograph of amygdaloid kindled rat. (after 5 days stimulation). Facial scratching (stage 2 manifestation) is evidenced.

Plate 13: Photograph of amygdaloid kindled rat. (after 7 days stimulation). Fore limb clonus (stage 3 manifestation) is evidenced.

Plate 14: Photograph of amygdaloid kindled rat. (after 14 days stimulation). Rearing posture (stage 4 manifestation) is evidenced.
voltage slow waves or α waves (Plate 15 (A)). These rats showed no abnormalities in their behaviour pattern. All rats injected PTZ (i.p.) and PCN (intracortically) and amygdaloid kindled developed epileptic EEG activity. The animals showed spontaneous and synchronous epileptic discharges in their ECoGs of ipsilateral and contra lateral foci and consisted of spikes and spike-wave complexes.

**In acute animals**

After intracortical injection of penicillin all the rats developed spontaneous synchronous epileptic discharges marked with bursts, spikes and multiple spike wave complexes. In acute animals, the basic pattern of the normal electrical activity was changed within a few seconds; first there was increase in the frequency for 1-2 minutes followed by diminution in the amplitude of the waves. At first isolated spike discharges were observed which gradually built up into high voltage discharges. During this period the background electrical activity showed increased α burst, both in amplitude and in frequency.

![BEHAVIOURAL PATTERN OF AMYGDALOID KINDLED EPILEPTIC RATS](image)

**Fig. 4.3**: Gradual kindling development induced by electrical stimulation of the basolateral nucleus of amygdala in rats. Each value represents the Mean ± SEM of eight rats. The seizure stages were evaluated using the 5-stage scale of Racine (1972). Scores: 1 = Facial clonus, 2 = Facial clonus and rhythmic head nodding, 3 = Facial clonus, head nodding and fore limb clonus, 4 = Facial clonus, head nodding, fore limb clonus and rearing, 5 = Facial clonus, head nodding, fore limb clonus rearing and falling
During this time spikes could be triggered very easily by photic stimulation. The epileptic pattern of the seizure activity developed gradually within 10-15 minutes. At first there were isolated sharp waves (Plate 15 (B)), then outburst of sharp waves followed by spikes of progressively higher voltage and finally bursts of poly spike appeared. This stage of spiking was later on followed by paroxysmal afterdischarges of shorter or longer durations. The spikes were initially positive, later on became biphasic -positive negative. Once this rhythmic afterdischarge was developed, it usually appeared at regular intervals but mostly limited on the ipsilateral hemisphere. At this phase occasional twitching and no clonic movement was clinically appreciable.

After a number of repetitions, these afterdischarges progressively increased in duration and spread both anteriorly and contralaterally until the whole brain became hyperexcitable as evidenced by paroxysmal high voltage discharges from all around the hemisphere of both sides. This gradual spread of epileptic activity to the contralateral hemisphere was accompanied by unilateral and bilateral clonic movements and twitching. The intensity of the discharges increased gradually both in frequency and amplitude and built up into synchronous high voltage generalized seizure discharges within 70-95 minutes (Plate 15 (C)). Each of these generalized seizure episodes lasted for about 15 seconds to 4 minutes and recurred frequently followed by a period of cortical silence. The inter-seizure period gradually lessened from 30 minutes to 2-5 minutes. Thus the sequence of intermittent, multiple fast spiking with gradual increase in intensity and duration of spike discharges followed by generalized seizure discharges all over the brain was seen in all the acute epileptic animals prepared by topical application of penicillin. The intensity, duration and spread of such discharges in
Plate 15: Representative electroencephalographic recordings showing the time course of the development and build up of seizure activity induced by penicillin in rats.

A. Record from control in which saline was injected at the somato sensory cortex.

B. Appearance of high voltage spike waves 15 minutes after PCN application in somato sensory cortex.

C. EEG showing typical generalised seizure in acute (95 minutes Post PCN injection) epileptic rat.
areas gradually altered its nature and pattern within 4-6 hrs following penicillin application.

During the inter seizure phase the animals became highly responsive to any type of sensory stimulation either mechanical (pinching), photic or auditory stimulation. Repeated clapping resulted in an increase of sharp waves and spikes along with focal or generalized seizures.

**In chronic animals**

In the chronic PCN epileptic animals, the background activity showed slow waves of high voltage with progressive increase in frequency. The immediate changes in the clinical and electrocortical manifestations were similar to those of the acute animals. These manifestations persisted for 3-4 weeks in the chronic group whereas in the acute preparations the clinical convulsive motor phenomenon did not prolong beyond 24-48 hours and the recurrence of tonic clonic convulsive seizure subsided within 3-7 days. 15 days after PCN application gradual development of seizure discharges could be provoked by photic stimulation (Plate 16 (A)). 30 days after PCN application EEG recordings were marked with spike discharges of very low amplitude (Plate 16 (B)) and there was no spontaneous generalized epileptiform discharge. However chronic epileptic animals even after 3-4 weeks could be provoked by pentylentetrazol stimulation. The paroxysmal high voltage spike activity appeared within 30 seconds from the different areas of the cerebral cortex and had no focal tendency although some irregular isolated bursts were seen from the site of injection.

**Electroencephalographic changes in PTZ induced epileptic rats:**

First there was increase in the frequency for 1-2 minutes followed by lowering of amplitude of the waves. The isolated spike discharges observed in the beginning gradually built up into high voltage discharges. The epileptic pattern of myoclonic
petitmal seizure developed within 10-20 minutes of PTZ administration (Plate 17). EEG recordings showed discharges with spikes, waves and domes followed by spreading depression when individually reaching seizure stage 2 or 3. Each of these generalized seizure episodes lasted for about 15 seconds to 2 minutes and recurred frequently followed by a period of rest. The inter-seizure period gradually lessened from 20 minutes to 5-8 minutes. The intensity, duration and spread of such discharges gradually altered within 3-5 hours after PTZ administration. Like the PCN induced epileptic during the inter seizure phase the animals became highly responsive to any type of sensory stimulation either mechanical (pinching), photic or auditory stimulation. Repeated clapping resulted in an increase of sharp waves and spikes along with generalized seizures.

**Electroencephalographic changes in amygdaloid kindled rats:**

No epileptiform activity could be observed in the control rats before amygdala kindling (Plate 18 (I)). Amygdaloid stimulation elicited focal electrical seizure activity with an EEG afterdischarge pattern with no initial visible seizure. Spontaneous EEG discharge pattern as well as the type, timing and duration of EEG and behavioural seizure activity resembled that seen after stimulus – evoked focal versus generalized seizures during kindling. After 7 days of stimulation the cerebral cortex and amygdala showed greatly increased recurrent bursts of epileptiform activity consisting of spikes (Plate 18 (II)). Daily stimulation at the basolateral amygdala of all the animals produced progressive development of AD (Plate 19 (I)). Seizure discharges were observed in amygdala and the frontal cortex from the second day. The chronological changes in the duration of amygdaloid AD was measured until the first generalized convulsion. The number of kindling trials required to achieve the first generalized convulsion was determined and the duration of convulsive fits were quantified in each animal. Slight
Plate 16: Representative electroencephalographic recordings showing the seizure activity induced by penicillin in rats.

A. EEG showing isolated sharp wave discharges (15 days post PCN application). Gradual development of seizure discharges provoked by photic stimulation (↑) is to be noted.

B. 30 days after PCN application EEG recording is marked with spike discharges of very low amplitude.

Plate 17: Representative electroencephalographic recordings showing seizure activity induced by pentylenetetrazol administration in rats.

A. Sample corticographic record from sham control rats.

B. EEG tracing showing the effect of PTZ eliciting immediate seizure manifestation marked with waves domes and spikes.
Plate 18: Representative electroencephalographic recordings showing the time course of the development and build up of seizure activity induced by amygdaloid kindling in rats.

I. EEG recording from the cerebral cortex (CC) and basolateral nucleus of amygdala (BLA) of control rats before amygdala Stimulation.

II. After 7 days of stimulation the CC and BLA showed greatly increased recurrent bursts of epileptiform activity consisting of spikes.
ADs at the amygdala (not more than 10 sec duration) were observed in each rat from the first day stimulation for kindling (after determining the supramaximal intensity of stimuli) when the mouth movement were observed (stage 1). After second to fourth trials (2\textsuperscript{nd} to 4\textsuperscript{th} day), the duration of ADs lasted for more than 15 sec, with subsequent repeated trials, the duration of ADs elongated concomitantly with an increased amplitude (stage 2: rhythmic movement of the head and nodding). Stage 3 was reached in which foreleg clonic movement occurred and the duration of AD appeared to be more than 30 sec. Stages 4 and 5 were considered by generalized fit with clonic and tonic convulsions, when the AD duration varied from 40-60 sec or more. 15 days after kindling EEG recordings were still marked with generalized ADs. In the 30 days post-kindling rats both the amplitude and frequency of the ADs were lowered (Plate 19 (II)).
Plate 19: Representative electroencephalographic recordings showing the development of after discharge activity induced by amygdaloid kindling in rats.

I. EEG recording showing cortical (CC) and amygdaloid (BLA) after discharges in 15 days post kindled rats. Progressive development of after discharges from rhythmic spiky activity can be noted in BLA.

II. EEG recording showing after discharges of low amplitude in 30 days post kindled rat.