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REVIEW ARTICLE

METHODS AND CONSIDERATIONS FOR EXPERIMENTAL EVALUATION OF ANTIEPILEPTIC DRUGS

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Abstract : Research into epilepsy and development of antiepileptic drugs relies heavily on studies in experimental animals. Though the conventional pentylenetetrazole-induced seizures and electroshock-induced seizures remain the mainstay of any antiepileptic drug screening protocol, considering the diversity of seizure types and underlying pathologies encountered in epilectics, numerous other seizure models have been developed. Some of these experimental models of seizures and considerations governing their selection are reviewed.

Key words : seizures, screening, experimental models, anticonvulsants

INTRODUCTION

Epilepsy is one of the most common disorders of the central nervous system, worldwide. Though, the advent of newer techniques in neurobiology has provided some insight into the pathophysiology of epilepsies, many aspects of this phenomenon still remain unknown. It is, therefore, not surprising that the currently used antiepileptic drugs fail to provide satisfactory seizure control for nearly 15 to 20% patients with epilepsy especially those of partial epilepsies (1). For such patients, combinations of antiepileptic drugs are often prescribed in attempts to improve seizure control. However, toxicities associated with these drugs can further compromise quality of life while drug-drug interactions may complicate clinical management.

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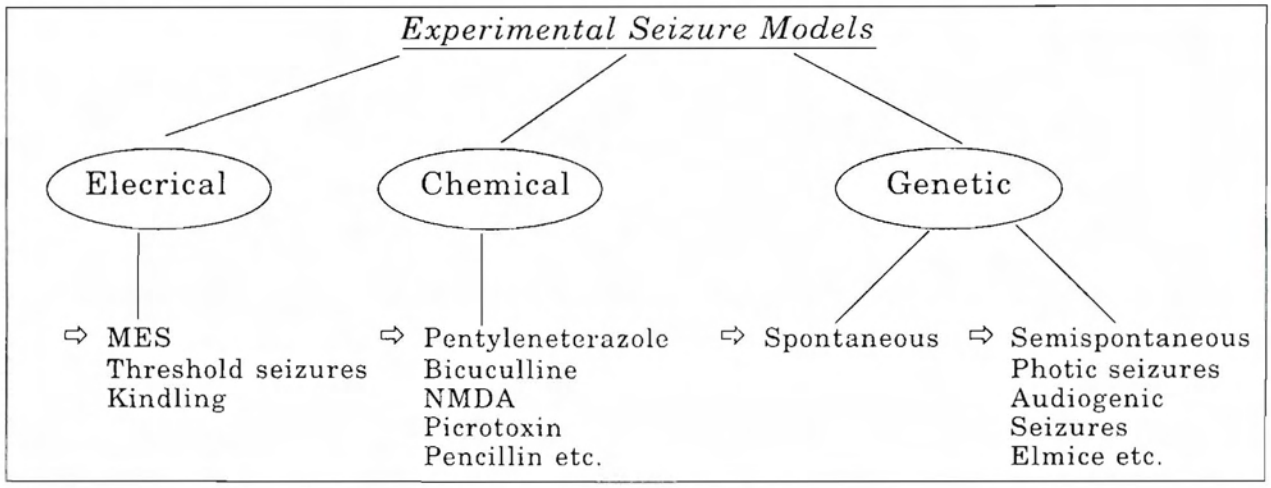

Fig. 1: Classification of experimental seizure models.

Thus there is an ever increasing need for research into the pathophysiology of epilepsy and newer molecules for treating epileptic seizures. For this suitable animal experimental models of seizures are required.

Experimental models used for seizure studies

Innumerable *in vitro* and *in vivo* models of seizures have been described over the years. The *in vitro* models include brain slices, monosynaptic systems and neuronal cultures. These are however, more suited for studying the specific epileptogenic mechanisms eg. paroxysmal depolarizing shifts, post-tetanic and long term potentiation, inhibition of GABA and glycine responses and spontaneous repetitive firing etc (2).

The *in vivo* models on the other hand employ diverse animal species like mice, rats, guinea pigs, gerbils, cats, dogs, monkeys etc. and use different physical and chemical/pharmacological stimuli to induce seizures. Some of these animal models of seizures are discussed.

An Ideal Animal Model of Seizures

Most animal models can not mimic all the pathophysiological, behavioural, electrophysiological and neurochemical alterations of the spontaneous human epileptic syndrome. Therefore, while a select few are used for routine screening of anticonvulsant compounds, some other help characterize or effect an understanding of mechanisms underlying epileptogenesis. In case of antiepileptic drug development, once a compound appears promising, a battery

TABLE I : Characteristics of ideal model of seizures.

1.	Development of spontaneously occuring recurrent seizures.
2.	Seizure type similar in clinical phenomenology to those in human epilepsy.
3.	Age-dependent onset of epilepsy as in generalized epileptic syndromes in man.
4.	Clinical seizures should be accompained by epileptiform activity in the EEG.
5.	Pharmacokinetics of antiepileptic drugs should be similar to those in humans.
6.	Effective plasma concentration of antiepileptic drugs similar to those required for controlling the particular seizure type in humans.

of tests is carried out to characterize the clinical profile and possible mechanisms of action. The characteristic features for an ideal model of seizures are listed in Table I.

Types of Animal Models of Epilepsy

The models available for screening drugs for antiepileptic activity may be broadly classified under three headings – a) electrical models, b) chemical models and c) genetic models (Fig. 1).

Electrically – induced seizures

Electrically–induced seizures have three variants namely, maximal electroshock, (MES) test, threshold models and kindling (Fig. 2).

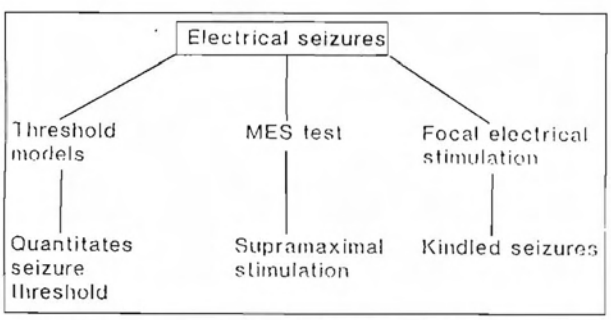

Fig. 2: Types of Electrical Seizures

In the **maximal electroshock (MES)–induced seizures,** the animals used are mice or rats. An electrical stimulus of sufficient intensity to induce maximal (tonic extension) seizures of the hind limb is applied, by means of an external device stimulator/convulsiometer. A supramaximal current strength, i.e. a stimulus about 5–10 times higher than the individual electrical seizure threshold of the animals is used (50 mA in mice or 150 mA in rats and for 0.25), (3, 4). The stimulus is applied via corneal or ear clip electrodes. Drugs like phenytoin, carbamazepine, phenobarbitone and primidone are highly active in this test while ethosuximide is ineffective. A big limitation of this test is that the stimulus is very strong. Because of this, some potentially useful agents may be missed. To obviate this threshold seizures are employed. However, MES seizures remain the primary screen for potential antiepileptic activity.

In **threshold test,** the ability of a drug to alter the seizure threshold for tonic hind limb extension is determined as, the current or voltage inducing hind limb extension in 50% of the animals (5). This test is a better predictor of generalized seizures of grandmal type and is much more sensitive to drugs than the MES test. Furthermore, threshold tests allow detection of proconvulsant effects of a given drug.

In **kindling,** repeated administration of an initially subconvulsive electrical stimulus results in progressive intensification of seizure activity, culminating in a generalized seizure (6, 7). The modification induced is robust and permanent. The seizures evolve through five stages – 1) immobility, eye closure, twitching of vibrissae, 2) facial clonus and head nodding 3) unilateral forelimb clonus (contralateral to the focus), 4) rearing often accompanied by bilateral forelimb clonus, 5) rearing and falling accompanied by generalized clonic seizures. Stages 1 and 2 correspond to complex partial (limbic or temporal lobe seizures) while stages 3 to 5 signify limbic seizures evolving to generalized motor seizures (7). Kindling is a relatively time consuming procedure, requires chronic implantation of stimulation and recording electrodes and regular electrical

stimulation. The advantage of this model is that the efficacy of drug against the progressive process leading to epileptogenesis as well as against the fully kindled state can be determined (8). Many antiepileptic drugs block kindled seizures while others are effective against development of kindling. Thus phenobarb-ital, diazepam and valproate block kindled seizures and development of kindling process. Phenytoin and carbamazepine block seizures once kindling has occurred but do not reliably block establishment of kindling (9).

In addition, in some species, the ictal phenomenon of partial epilepsy can be reproduced by acute focal electrical stimulation with convulsive stimuli ie. electrically –induced localized cortical, amygdalar, thalamic or hippocampal after discharges, in monkeys, dogs and cats (10). However, unlike kindling, in this case, the epileptogenic progress can not be studied.

Genetic models of Epilepsy

By definition of epilepsy, animals with chronically recurring, spontaneous seizures represent ideal models for human epilepsy. Some species and strains typically and repeatedly exhibit spontaneous seizures, or, have an extremely low threshold to a number of epileptogenic agents such as stress and sensory stimulation. These are the genetic models of epilepsy. The genetic models may be classified into two groups. 1) Spontaneous, and, 2) Semi spontaneous.

Spontaneous seizures

Beagle dogs show a high incidence of epilepsy including secondary forms and two types of primary convulsion resembling complex partial seizures and generalized tonic – clonic seizures (11).

In mice there are at least 12 single locus mutations that produce neurological syndromes with spontaneous seizures (12). These include the **homozygous tottering mouse, quaking mice, AE mice and C57BL/10Bg strain** (13). Some rats with spontaneously occurring spike –wave petit mal discharges have also been observed. Myoclonic and generalised tonic seizures also occur both spontaneously and in response to mild stress in the inbred line BIO 86. 93 of **syrian hamsters** (8).

The spontaneous models for seizures may be ideal for chronic drug efficacy studies once, a drug has been shown to be a valuable candidate for further development. For acute antiepileptic drug efficacy studies however, none of the animal species with spontaneously occurring seizures except rats with spike – wave petit mal seizures is useful. This is primarily due to logistical problems such as availability of the animals and/or too low or irregular seizure frequency of the spontaneous seizures which necessitates artificial induction of seizures.

Semispontaneous seizures

In certain genetically predisposed species, seizures do not occur spontaneously but, can be induced by specific sensory stimulation, such as audiogenic or photic stimuli.

Photic seizures

Myoclonic responses to intermittent photic stimulation occurs is 60–80% of

adolescent baboons, *Papio papio* (14). The sensitivity of this response to antiepileptic drugs is however only in part similar to human syndromes. Photically-induced seizures also occur in domestic fowls (8). These seizures are inhibited by drugs useful against clinical tonic-clonic and myoclonic epilepsy, including benzodiazepines, barbiturates and valproic acid. Less favourable therapeutic effects are seen with phenytoin, carbamazepine and trimethadione (9). These seizure models have certain drawbacks like – a) Predictive value against a particular clinical subtype of seizures is questionable and, b) high prime and maintenance cost of baboons limits its usefulness.

Audiogenic seizures :

Mice with an enhanced susceptibility to audiogenic stimulation have been seen. The DBA/2J inbred strain of house mouse *Mus musculus* is highly susceptible (15). It exhibits severe sound-induced seizures between age 2 and 4 weeks after which susceptibility gradually declines. However, at 8 weeks of age, when DBA/2J mice are free from seizures, they show a low threshold to seizures induced by electroshock or excitatory amino acids (16). Another strain SJL/J on the other hand develop less severe seizures after a few days of priming exposure to sound (17). The seizures comprise of initial startle followed by running and leaping. In some cases clonic jerks, prolonged tonic spasms and death may also occur. Audiogenic seizures are prevented by phenytoin, phenobarbital or valproate.

Sound induced convulsions also occur in rats. The seizures in genetically-epilepsy-prone rats (GEPR) are similar to those in audiogenic seizures-susceptible mice (15). However, while GEPRs may be a valuable alternative to traditional MES test, seizures in mice are not sensitive to a specific clinical category of antiepileptic drugs but may be useful as a gross screen for potential antiepileptic drugs.

The major drawback with such seizures is that sound-induced seizures are exceedingly rare in man and questions as to correlation.

Totterer mouse :

Totterer mouse (tg/tg strain) has hereditary ataxia, myoclonus and frequent partial and absence seizures (18, 19). This is accompanied by 6–7/s spike wave EEG changes (20).

Mongolian gerbil :

In case of mongolian gerbil, seizures can be precipitated by environmental stimuli like onset of bright light, audiogenic stimuli, vigorous shaking of cage, different handling techniques and or air blast (average pressure 5–10 bars, directed at the back of the animals for 15 sec.) (21). The seizures range in severity from facial myoclonic 'major' and generalized myoclonic to tonic-clonic 'major' seizures (22). Drawbacks of this model are the following – (a) non specificity of sensory stimulation for epileptic myoclonic seizures (b) experiments are time consuming, (c) skill in handling mandatory to avoid induction of seizures, (d) the half life of drugs is shorter than in humans and maintenance of effective levels during chronic treatment difficult.

El mice :

El mice is a mouse mutant suited as an animal model for complex partial seizures. Seizures are induced in response to vestibular stimulation ie, by repeated tosses into the air or by altering the equilibrium of the mice (12). This model is also not used widely for antiepileptic drug evaluation but is primarily employed in search for seizure mechanisms.

More recently a strain of rats with spontaneous absence like and tonic clonic seizures has been described. These are the 'tremor' homozygous rats and 'zitter' homozygous rats. These spontaneously epileptic rats (SER) exhibit 5–7/s spike wave like complexes in cortex and hippocampus during periods of abnormal immobility. Ethosuximide, trimethadione, valproate and phenobarbital inhibit absence seizures and phenytoin, valproate and phenobarbital inhibit tonic seizures in the SER rat (23).

Drosophila shakers :

A mutant fly, named 'shaker', has been observed to shake and flutter when exposed to ether. Intracellular recordings from flight muscles indicate deficits of 'A current'. The absence of this current would be expected to result in prolonged firing of excitable tissue. Genetic studies are underway to determine a link between seizure like behaviour with a characterized physiologic and genetic deficit. Whether such a genetic abnormality of potassium channels occurs in humans is unknown (9).

Thus genetic models of seizures are better suited for obtaining information about the fundamental mechanisms involved in the onset and development of epilepsies rather than anticonvulsant screening. In the Indian setup, availability is major limitation with these models.

Chemically-induced seizure models

Innumerable chemicals and drugs induce seizures at toxic doses and many of them can be used to produce epileptiform phenomena in experimental animals. The chemoconvulsants may be administered either systemically or topically i.e. central

TABLE II : Chemical models of seizures.

A. Systemic	**B. Central**
1. Pentylenetetrazole	1. Penicillin
2. GABA antagonists-bicuculline, picrotoxin, penicillin	2. Kainic acid
3. GABA synthesis inhibitors–INH, D–penicillamine	3. Quinolinic acid
4. GAD antagonists 3–Mercaptopropionic acid	
5. Inverse benzodiazepine agonists–DMCM, FG 7142	
6. Glycine antagonist-Strychnine	
7. Cholinomimetic drugs-Pilocarpine	
8. EAA agonists-NMDA, Kainic acid	
C. Topical convulsants	
1. Alumina cream	
2. Cobalt	
3. Tungstic acid	
4. Premarin	

(focal) application (Table II). While inhibition of GABAergic transmission by action at different loci is the basis of action of most systemic convulsants like pentylenetetrazole (PTZ), bicuculline, picrotoxin etc., other alternate mechanisms have also been identified. These include benzodiazepine inverse agonists, like DMCM (Methyl–6, 7–dimethoxy–beta–carboline 3–carboxylate); cholinomimetic drugs like pilocarpine; excitatory amino acid receptor agonists-N-methyl-D-aspartate and kainic acid; glycine antagonist-strychnine etc (8). In addition, certain metals including cobalt, iron and alumina may be applied to cortical areas to induce syndromes of chronic focal seizures in mammals i.e. rats, cats and monkeys (10).

Pentylenetetrazole (PTZ) as convulsant :

The systemic administration of pentylenetetrazole (PTZ) is one of the most commonly employed method for anticonvulsant screening. The obvious reasons for this preference are the ease of performance, reliability, ease of expressing results and the shorter time needed to produce convulsions. Pentylenetetrazole (Pentamethylenetetrazole, metrazol, cardiazol, leptazol), was introduced for anticonvulsant screening by Richard and Everett, (24), when they demonstrated the unique protective ability of trimethadione to prevent seizures which it evoked. For inducing seizures in rats, the dose of PTZ suggested in literature varies from 70 to 90 mg/kg. However, dose effect measurements and calculation of the CD_{97} i.e. the dose of PTZ causing clonic seizures in 97% of the animals is carried out prior to anticonvulsant drug evaluation by the PTZ test (25). PTZ 60 mg/kg has been found to produce generalized clonic seizures in 100% animals in our laboratory setup. This particular does has the advantage of causing least mortality (26).

PTZ induces generalized clonic and, in higher does tonic seizures after different routes of administration i.e. subcutaneous or intraperitoneal to rats, mice, cats, and primates. The seizures are paralleled by spike wave complexes (clonic seizures) or sharp hypersynchronized polyspikes (tonic seizures) in the EEG.

The mechanism of convulsant action of PTZ seems to be related to the inhibition of the inhibitory functions of the GABA neurotransmitter. PTZ has affinity for the chloride-ionophore of the postsynaptic GABA receptor complex and to antagonize GABAergic function (27). Although several other compounds used like bicuculline, picrotoxin, benzylpenicillin also impair GABAergic neurotransmission, the site of action is different ie. antagonistic action at GABA receptors. Furthermore, the seizures induced by these compounds are in most instances similar to those induced by PTZ so that these chemoconvulsants do not exhibit advantages vs. PTZ for drug screening.

The end points used by different researchers in the PTZ seizure model, differ. While some have used the first generalized clonic seizure with loss of righting reflex as endpoint (28, 29), Swinyard (4) has proposed using a threshold seizure i.e. the first episode of continuous generalized clonic seizure with loss of righting reflex for at least 5 seconds as end point.

Likewise, different indices of anticonvulsant activity have been used. These include incidence and latency of the different seizure types (30, 31). Alternatively, comparisons of efficacy are made based on severity of seizures determined by an arbitrary scoring system (32). In addition to clonic seizure end points, drug effects in tonic seizures can be studied by using a higher PTZ dose which induces tonic seizures in all the animals (33, 34). Ethosuximide, valproate and benzodiazepines are active in this test while, phenytoin and carbamazepine are not (8, 35). Phenobarbitone and primidone block PTZ seizures much more potently than ethosuximide and valproate. This might suggest that PTZ is a model for myoclonic rather than petit mal seizures.

PENTYLENETETRAZOLE (PTZ)-INDUCED KINDLING IN RATS

Kinding can also be induced in rats and mice by repeated injections of small doses of PTZ. This PTZ kindling is regarded as a good animal model of generalized epilepsy (36). In our laboratory, we have observed that administration of subconvulsant dose of PTZ 30 mg/kg, ip, every alternate day, three times a week for nearly ten weeks leads to development of kindled seizures in rats. These seizures are blocked by diazepam and sodium valproate (37). The seizures induced are characterized by generalized spike and wave discharges on the EEG concomitant with generalized seizures such as myoclonic and tonic seizures.

Though PTZ is known to act as a potent convulsant by acting via a specific interaction with GABA–coupled chloride ionophore (38), however, there is little evidence for the basic neuronal mechanism of the PTZ–kindled seizures. It is conceivable that chronic treatment with PTZ may bring about certain changes in the neurotransmitter milieu as well as their receptors and effectors. Not only this, different neuronal mechanism for PTZ kindling and amygdaloid kindling have been suggested (39).

Bicuculline :

Bicuculline is a GABA antagonist and has been applied both systemically and focally to induce seizures. In another model known as 'systemic focal epileptogenesis', the rats receive radiation to a limited volume (0.25 ml) of cerebrum. Three to six months later, when the blood-brain barrier is locally disrupted, bicuculline methiodide (2 mg/kg), is injected systemically. As such bicuculline does not cross the blood-brain barrier but in this case due to the disrupted barrier, an epileptic focus with recurrent EEG spikes and focal seizures enduring for several weeks are induced. The spikes are suppressed by phenytoin, phenobarbital, chlordiazepoxide and valproic acid (40).

The convulsant action of bicuculline is related to competitive inhibition of GABA binding at the receptor and consequent block of GABAergic neurotransmission.

Picrotoxin :

Picrotoxin has a mechanism of action similar to bicuculline and in general the antiepileptics are similarly effective against picrotoxin and bicuculline seizures. The exception is carbamazepine which has some benefit against picrotoxin but not bicuculline seizures (41). Phenytoin is ineffective against both types of seizures

while diazepam is highly effective. Phenobarbital has intermediate effectiveness and the antiabsence agents valproate and ethosuximide have low effectiveness (41, 42).

β-Carbolines :

Convulsant and proconvulsant actions of β-carbolines such as ethyl-β-carboline-3-carboxylate (β-CCE), methyl-β-carboline-3-carboxylate (β-CCM) and methyl-6, 7-dimethoxyl-4-ethyl-β-carboline-3-carboxylate (DMCM) are well documented. β-CCE enhances convulsions in rodent and baboon epilepsy models (43, 44) while β-CCM 0.03 mg/kg, intravenous produces seizures in epileptic chickens (45). In mice β-CCM, subcutaneously, induces convulsions and these convulsions are inhibited by benzodiazepine agonist diazepam and the partial agonist Ro 15-1788 (46). DMCM also has potent convulsant properties (47). The mechanism of action appears to involve interaction with the benzodiazepine receptor and consequently with $GABA_A$ receptor (48).

Gama-hydroxybutyrate (GHB) model :

GHB is a naturally occurring metabolite of gamma-aminobutyric acid (GABA), which when given to animals, produces electroencephalographic (EEG) and behavioural changes, resembling generalized absence seizures (49-51). Characteristically, the GHB-treated animals, both monkeys and rats, show an arrest of activity with staring and bilaterally synchronous spike wave discharges. This activity is selectively blocked by antiabsence drugs such as ethosuximide and made worse by phenytoin (52). A prodrug of GHB, gamma-butyrolactone (GBL) has also been shown to induce spike and wave discharges, by being rapidly converted to GHB on parenteral administration. The EEG and behavioural changes induced are similar to those by GHB rather they have a more rapid onset of action and predictable dose response. GBL *per se* has no such effect (53). More recently, it has been reported that rats develop spontaneously occurring recurrent electroclinical seizures after repeated daily injections of GBL (54). The mechanism of epileptiform activity remains elusive but an interaction with some neurotransmitter system or more specifically inhibition of GABAergic neurotransmission is a possibility (55, 56).

Excitatory amino acids as convulsants

N-methyl-D-aspartate (NMDA) :

Excitatory amino acids cause convulsions when administered into the brain. Of the various excitatory amino acid receptors, a specific role for the NMDA receptor in epilepsy has been proposed. NMDA receptor density is highest in hippocampus, followed by neocortex, striatum and thalamus. Intracerebroventricular administration of NMDA in mice induces running fits, generalized clonic and tonic-clonic convulsions. These seizures are inhibited by diazepam, valproate, several NMDA receptor antagonists like MK-801, but not by midazolam, clonazepam, phenytoin, carbamazepine, trimethadione and ethosuximide (57).

Kainic acid :

Kainic acid (KA) is an analog of glutamate, an excitatory amino acid

neurotransmitter. Systemically or centrally administered KA has toxic effects on hippocampus producing cell injury, while in lower doses, seizures are induced (58). After, ip administration of KA, in rats or mice, the convulsive behaviour comprises of wet dog shakes, staring, searching and gnawing, leading to hyperactivity, forelimb clonus and tonic clonic convulsions (59, 60). Kainate induced seizures have also been reported in cats (61). These kainate induced seizures are proposed to be a model with particular relevance to human temporal lobe epilepsy (62). The mechanism involved may be disinhibition or direct excitation. The KA seizures are abolished by phenobarbital, midazolam, clonazepam and valproate (57). Kainate induced seizures have also been used as a model of status epilepticus and will be discussed later.

Tetanus toxin :

Tetanus toxin was first used in 1962, to induce epileptiform activity by application on cerebral cortex in dogs (63). Ever since, it has been used successfully as a convulsant in several species ie. rat, dog, cat and in several brain regions (2). In the mouse, after hippocampal injections, seizures may occur within a day after injection and then recur for weeks. The animal sequentially shows arrest of activity, myoclonic jerks of forelimbs and sometimes generalized tonic-clonic seizures (64). Electroencephalographically, a 3–20 Hz spiking or spike-wave activity is observed. The seizures are taken to correspond to complex partial seizures and are believed to be related to site of injection. The mechanism involved is unknown however, blocking of presynaptic release of GABA and glycine could be involved (65).

Penicillin :

The convulsant action of penicillin was discovered by Walker and Johnson (66). Topically applied penicillin is the most popular method to study simple partial (focal) seizures. Recurrent interictal spikes are observed when a cottonoid pledget soaked in 1.7–3.4 mM penicillin is placed on exposed rat or cat cortex.

Penicillin has also been employed in models of systemic focal epileptogenesis and models of generalized tonic-clonic and absence seizures. Thus parenteral administration of penicillin ($\geq$300,00 units/kg, im) in rats and cats, produces recurrent episodes of arrested activity, staring, myoclonous, facial-oral twitching and occasional progression to generalized tonic-clonic seizures (67, 68). Epileptic activity begins about 1h after injection and continues intermittently for 6–8 h (69). The convulsive action is due to competitive binding at the GABA receptor. Consistent with its profile as a model for absence seizures, valproate and ethosuximide are more effective than phenytoin in these seizures (70, 71).

Metals

Metals are implanted in brain to generate a state of 'spontaneously' recurrent simple partial seizures. Various metals are used for the purpose eg. cobalt, tungsten, zinc, iron and alumina. Of these, the aluminium hydroxide gel model discovered by Kopeloffs (72), is the most well characterized. In this, 4% aluminium hydroxide gel is injected into the surgically exposed cat or monkey neocortex at a couple

of adjacent sites. Seizures are initiated after 1–2 months and may persist for years. The seizures consist of rhythmic jerking of an extremity or face contralateral to the lesion and may occasionally generalize.

In addition to these well established models, seizures are often induced by methods like cryogenic injury or freeze lesion model, ganglioside antibody injection and metabolic derangements. In *freeze lesion model,* ethylchloride lesions or cold trauma from a liquid nitrogen probe induces an epileptic focus (73, 74). In *ganglioside antibody injection,* epileptic lesions are produced by injection of antibodies to brain gangliosides into rat cortex. Focal spiking occurs after a delay of 24 h and may recur till 90 days (75, 76). Seizures may also be produced by certain types of *metabolic derangements* eg. hypoglycaemia, uremia, hypoxia etc (9, 10). Administration of methylxanthines in high doses induces seizures in mice and rats (77).

Status epilepticus

Status epilepticus (SE) is recognized as a major neurological and medical emergency associated with significant morbidity and mortality. It is defined as continuous seizure activity for more than 30 min, or intermittent seizure activity without regaining of consciousness lasting for more than 30 min (78). SE may be convulsive or non convulsive and these two types are further subclassified as partial or generalized.

Several *in vivo* animal models of SE have been developed, and the electrographic, histological and neurohumoral changes in these, parallel those in SE patients.

Electrically-induced SE :

Certain electrical paradigms have been shown to produce SE in rats. Thus, low intensity (60 min) electrical stimulation from a previously kindled amygdala or hippocampal focus induces SE (79). Accompanying behavioural manifestations may be convulsive as in kindled seizures or non convulsive, with normal stereotyped or arrest behaviours (80). Vicedomini and Naddler(81) induced SE using pulse trains (0.3 ms monophasic square wave pulses at 20 Hz, 10–s duration, 30s) with maximal synaptic response in CA3 region of hippocampus. Taber et al (82) produced a syndrome of recurrent seizures by repeated hippocampal stimulation in mice. McIntyre et al (80) have reported a self sustaining SE in previously kindled rats by continuous hippocampal stimulation (CHS). A self sustaining limbic SE by CHS, in non kindled rats has also been reported (83). George et al, (84) have described SE by continuous ventral hippocampal stimulation (decreasing the period of CHS from 9 to 5 epochs) followed by low dose, pilocarpine (40 mg/kg) challenge. The motor limbic seizures occurring were completely reversed by diazepam, clonazepam and MK–801.

Chemically-induced SE :

In rats, convulsive, generalized tonic-clonic SE can be modelled by administration of bicuculline (85), focal application of cobalt in conjunction with systemic homocysteine (86).

Kainic acid model :

Following injection of kainic acid, rats manifest severe limbic motor seizures and SE, approximately 90 min after (87). This SE ultimately results in extensive loss of several limbic structures, in particular the olfactory or piriform cortex and this is manifested as long lasting cognitive deficits (88).

Lithium-pilocarpine models :

Seizures elicited by the cholinergic muscarinic agonist pilocarpine in rodents have been proposed as an animal model, resembling some aspects of human temporal lobe or psychomotor epilepsy (89, 90). These seizures have been related to rapid evolution of hippocampal or amygdala kindling (91). Sequential injections of lithium salt and pilocarpine (separated by 20–24 hr) reliably induces SE in rats. Though in terms of behavioural and electrographic findings, lithium-pilocarpine induced seizures are not much different from high dose pilocarpine seizures, the former reproduces SE more reliably (92–94). Recently, we have shown that lithium 3meq, ip, given 4 hr prior to pilocarpine 30 mg/kg, s.c is an effective and reliable model for SE (95). The seizures evolve from cholinergic hyperactivity to full blown clonic seizures occurring repeatedly (Fig. 3). This treatment schedule has the advantage of convenience ie. the experiment can be finished the same day. These seizures are aborted by diazepam and the neuropathological changes are same as those with 20–24 hr pretreatment.

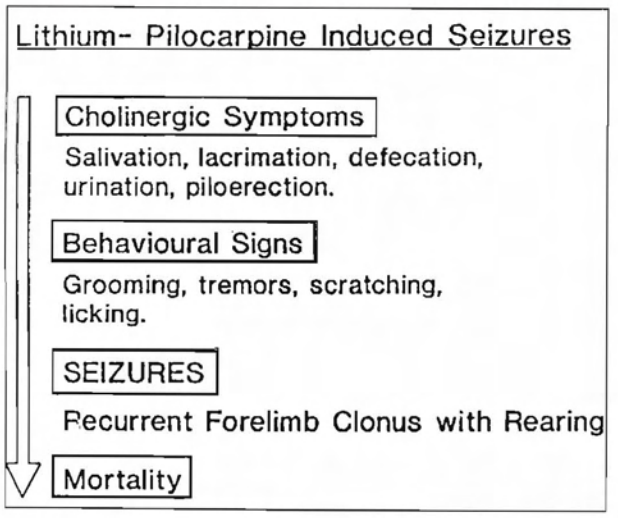

Fig. 3 : Development of Lithium-pilocarpine induced status epilepticus.

Post-traumatic Epilepsy

Any serious injury like major head trauma, stroke, hemorrhage etc. can cause epilepsy. Although a seizure can occur at the time of initial insult in some cases, in others, there may be a latent period of months to years for the onset of epilepsy. Some animal models have been developed to replicate this posttraumatic epilepsy. The most well characterized and widely studied of these is the ferric chloride ($FeCl_3$) model wherein, 100 mM of $FeCl_3$ administered into the ventricles induce electroencephalographic seizures (96, 97). Others are, intracortical injection of hemoglobin or ferrous chloride (98–101).

More recently, intracerebro-ventricularly administered endothelin has also been shown to induce seizures (102).

Although a number of animal species and numerous experimental models are available for screening of antiepileptic compounds, the selection of model can not be done arbitrarily. This is governed by some considerations.

Considerations in selecting an animal model

Seizure type :

Since different animal models are believed to mimic different types of seizures, the major consideration in selecting an animal model of course remains the seizure type under study. A possible classification of the different animal models based on the seizure type is given in Table III.

TABLE III : Animal models of epilepsies based on seizure type.

A. Generalized tonic-clonic
– Maximal electroshock (MES)
– Chemical convulsions Pentylenetetrazole, Picrotoxin, Bicuculline, Penicillin
– Genetic Photosensitive baboons, autiodenic Seizures in mice, Genetically epilepsy prone rats, Totterer and E1 mice, Drosophila shakers
– Metabolic derangements Hypoxia, hypoglycaemia, hyperbaric oxygen, uremia
B. Generalized absence
– Thalamic stimulation
– Systemic penicillin
– Gamma–hydroxybutyrate
C. Simple partial seizures
– Acute Topical convulsants–Penicillin, Bicuculline, Strychnine, Cholinergics, Picrotoxin Acute electrical stimulation
– Chronic Chronically implanted metals – Alumina, Cobalt, Iron, Zinc, Tungsten
D. Complex partial seizures
– Kainic acid
– Tetanus toxin
– Kindling
E. Status epilepticus
– Fluorothyl, bicuculline, penicillin
– Cobalt/Homocysteine
– Kainic acid
– Lithium – pilocarpine
– High dose pilocarpine
– Continuous hippocampal stimulation (CHS)
– Partial CHS–pilocarpine
– Kindling based
F. Post traumatic epilepsy
– Ferric chloride
– Hemoglobin

Species :

The second factor is animal species. Though, most convulsive stimuli actively produce seizures in many speices, there may be differences in drug response. Therefore, it is advisable to choose appropriate species. Generally, screening at initial stages is carried out with two species ie. rats and mice to avoid the possibility of missing out a potential agent.

Male versus female rats for anticonvulsant studies :

A relationship between seizure susceptibility and the estrous cycle is well known (103, 104). In studies in humans as well, a subset of women with epilepsy show changes in seizure frequency in relation to hormonal variations during the menstrual cycle (103, 104). Whanschaffe and Loscher, (105), on the other hand, have demonstrated no effect of setrous cycle on kindled seizures in female rats.

Thus with the use of female rats, there is always remaining at least a theoretical possibility of variation in response with the changing hormonal status of the animal. Furthermore, female rats are known to eliminate several antiepileptic drugs less rapidly than males do (32). This again is not unambiguous (105).

Selection of endpoint/observational parameters for evaluating anticonvulsant response :

The endpoints used by different researchers in various models differ. For eg as discussed earlier, in PTZ seizures different end points are used for assessing. This end point selection may at times be crucial in interpreting results especially in case of compounds with weak activities. The effect of end point selection, on drug potencies in PTZ seizures model has been examined, in a comprehensive study by Loscher et al (32).

Need for standardization :

The data reported by different laboratories on the commonly used antiepileptic drugs and those that are newly developed, at times, differs considerably both qualitatively as well as quantitatively. For instance, various studies found phenytoin, carbamazepine and primidone to be ineffective against clonic seizures induced by s.c. PTZ administration (106–108) or iv PTZ infusion (109). This would be consistent with their inefficacy in human absence seizures. On the contrary, there are several experimental studies also in which these drugs are found to be capable of blocking clonic PTZ seizures (110–112). This is difficult to explain by differences in dose or routes of administration of PTZ. The newly developed antiepileptic drugs eg. vigabatrin and progabide also were reported to be ineffective against clonic seizures induced by s.c. PTZ in mice (113), but other researchers reported both drugs to be effective (8, 112, 114). It is likely that in addition to recognized factors like species, strain, diet, sex, circadian, hormonal and seasonal rhythms, other technical, biological and/or pharmacological factors may be important (32). This however implies that the standardization of this test is far from being reached and thus there is need to standardize the PTZ-induced seizure model for the strain and species to be used and for a particular laboratory setup. This applies to other seizure models as well.

Observer bias :

The *in vivo* models used for studying anticonvulsant activity in rats and mice have largely been dependent on the continuous monitoring of the convulsive behaviour by an observer. This approach is tedious, and also there remains an unavoidable possibility of bias on the part of the observer and, the inter-individual variability, more with less experienced investigator. Thus it is desirable to obtain a hard copy of seizure activity.

Efforts have been made to develop simple techniques for quantitative measurement of clonic convulsions in rats. A method for automatically measuring clonic convulsions using capacitance sensors and digital counter, has been described by Paul and Kazi, (115). Their counter provides a measure of time course of the convulsive behaviour as a measure of anticonvulsive potential of test compounds. A 'Convulsometer' is also marketed by an International company, Columbus Instruments, U.S.A., which is rather costly. More recently we have designed a seizure recording assembly in our laboratory which offers an economical and practical alternative for continuously recording convulsive activity in rats and mice (116).

CONCLUSIONS

Studies in experimental seizure models have contributed vastly not only to the development of antiepileptic drugs but also has given some insight into the pathophysiology of epilepsies. It is therefore not surprising that many different experimental seizure models have evolved. These seizure models however do not mimic the actual disease but only some manifestations like electroencephalographic, behavioural, neurohumoral etc. It is noteworthy that evaluating a potential antiepileptic, using a single model of seizures is not sufficient. Rather, a battery of tests is required, to obtain the proper profile of the drug. Choosing the appropriate model and experimental paradigms is of paramount importance.

REFERENCES

1. Mattson RH. Drug treatment of partial epilepsy. *Adv Neurol* 1992; 57: 643–650.
2. De Deyn PP, Hooge RD, Marescau B, Pei YQ. Chemical models of epilepsy with some reference to their applicability in the development of anticonvulsants. *Epilepsy Res* 1992; 12: 87–110.
3. Loscher W, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. II. Maximal electroshock seizures. *Epilepsy Res* 1991; 8: 79–94.
4. Swinyard EA. Laboratory assay of clinically effective antiepileptic drugs. *J Am Pharm Assoc* 1949; 38: 201–204.
5. Swinyard EA. Electrically induced convulsions. In : D.P. Purpura, J.K. Penry, D. Tower, D.M., Woodbury and R. Walter (Eds), Experimental models of epilepsy–a manual for the laboratory worker, Raven press, New York, 1972; pp. 433–458.
6. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Exp Neurol* 1969; 25: 295–330.
7. Mc Namara JO. Kindling : an animal model of complex partial epilepsy. *Ann Neurol* 1984; 16 (Suppl.) : S72–S76.
8. Loscher W, Schmidt D. Which animal models should be used in the search for new antiepileptic drugs ? A proposal based on experimental and clinical considerations. *Epilepsy Res* 1988; 2: 145–181.
9. Fisher RS. Animal models of the epilepsies. *Brain Res Rev* 1989; 14: 245–278.
10. Koella WP. Animal experimental methods in the study of antiepileptic drugs. In : H.H. Frey and D. Janz (Eds.) Antiepileptic drugs. Handbook of experimental pharmacology, vol. 74, Springer-Berlin, 1985; pp. 283–339.
11. Loscher W, Schwartz-Porsche D, Frey HH, Schmidt D. Evaluation of epileptic dogs as an animal model of human epilepsy. *Arzneim-Forsch* 1985; 35: 82–87.
12. Seyfried TN, Glaser GH. A review of mouse mutants as genetic models of epilepsy. *Epilepsia* 1985; 26: 143–150.
13. Loscher W, Czuczwar SJ, Wolff GL. AE mice : an inbred mouse strain with interesting features for epilepsy research. *Epilepsia* 1986; 27: 657–664.
14. Killam EK. Photomyoclonic seizures in the baboon, Papio papio. *Fed Proc* 1979; 38: 2429–2433.
15. Loscher W. Genetic models of epilepsy as a unique resource for the evaluation of anticonvulsant drugs–A review. *Meth Find Exp Clin Pharmacol* 1984; 6: 531–547.
16. Engstrom FL, Woodbury DM. Seizure susceptibility in DBA and C57 mice : the effects of various convulsants. *Epilepsia* 1988; 29: 389–395.
17. Fuller JL, Collins RL. Temporal parameters of sensitization of audiogenic seizures in SJL/J mice. *Dev Psychobiol* 1968; 1: 185–188.
18. Kaplan BJ, Seyfried TN, Glaser GH. Spontaneous polyspike discharges in an epileptic mutant mouse (tottering). *Exp Neurol* 1979; 577–586.
19. Noebels JL, Sidman RJ. Inherited epilepsy : spike-wave and focal motor seizures in the mutant mouse tottering. *Science* 1979; 204: 1334–1336.

20. Noebels JL. A single gene error of noradrenergic axon growth synchronizes central neurones. *Nature (Lond.)* 1984; 310: 409–411.

21. Kaplan H. What triggers seizures in the gerbil, Meriones unguiculatus? *Life Sci* 1975; 17: 693–698.

22. Frey HH, Loscher W, Reichie R, Schultz D. Anticonvulsant potency of common antiepileptic drugs in the gerbil. *Pharmacology* 1993; 27: 330–335.

23. Sasa M, Ohno Y, Ujihara H, FujitaY, Yoshimura M, Takaori S, Serikawa T, Yamada J. Effects of antiepileptic drugs on absence like and tonic seizures in a spontaneously epileptic rat, a double mutant rat. *Epilepsia* 1988; 29: 505–513.

24. Richard RK, Everett GM. Analgesic and anticonvulsant properties of 3, 5, 5–trimethyloxazolidine 2, 4–dione (Tridione). *Fed Proc 1944; 3: 39*–44.

25. Swinyard EA, Brown WC, Goodman LS. Comparative assays of antiepileptic drugs in mice and rats. *J Pharmacol Exp Ther* 1952; 106: 319–330.

26. Malhotra J, Gupta YK. Effect of adenosine receptor modulation on pentylenetetrazole-induced seizures in rats. *Br J Pharmac* 1997; 120: 282–288.

27. Olsen RW. The GABA postsynaptic membrane receptor-ionophore complex. Site of action of convulsant and anticonvulsant drugs. *Mol Cell Biochem* 1981; 39: 261–279.

28. Frey HH, Loscher W. Di-n-propylacetic acid: profile of anticonvulsant activity in mice. *Arzneim Forsch (Drug Res)* 1976; 26: 299–301.

29. Killian M, Frey HH. Central monoamines and convulsive thresholds in mice and rats. *Neuropharmacology* 1973; 12: 681–692.

30. Osonoe K, Mori N, Suzuki K, Osonoe M. Antiepileptic effects of inhibitors of nitric oxide synthase examined in pentylenetetrazole-induced seizures in rats. *Brain Res* 1994; 663: 338–340.

31. Kubova H, Rathouska J, Mares P. Anticonvulsant effects of bretazenil (Ro 16–6028) during ontogenesis. *Epilepsia* 1993; 34: 1130–1134.

32. Loscher W, Honack D, Fassbender CP, Nolting B. The role of technical, biological and pharmacological factors in the laboratory evaluation of anticonvulsant drugs. III. Pentylenetetrazole seizures models. *Epilepsy Res* 1991; 8: 171–189.

33. Desmedt LKC, Niemegeers CJE, Janssen PAJ. Antagonism of maximal metrazol seizures in rats and its relevance to an experimental classification of antiepileptic drugs. *Arzneim Forsch (Drug Res)* 1976; 26: 1592–1602.

34. Piredda SG, Woodhead JH, Swinyard EA. Effects of stimulus intensity on the profile of anticonvulsant activity of phenytoin, ethosuximide and valproate. *J Pharmacol Exp Ther* 1985; 232: 741–745.

35. Malhotra J, Seth SD, Gupta SK, Gupta YK. Adenosinergic mechanisms in anticonvulsant action of diazepam and sodium valproate. *Env Toxicol Pharmacol* 1996; 1: 269–277.

36. Ono J, Vieth RF, Walson PD. Electrocorticographical observation of seizures induced by pentylenetetrazole (PTZ) injection in rats. *Functional Neurol* 1990; 5: 345–352.

37. Gupta YK, Malhotra J. Effect of theophyiline on diazepam and sodium valproate protection in pentylenetetrazole–kindled seizures in rats. *Indian J Physiol Pharamcol* 1997; 41: 280–284.

38. Corda MG, Orlandi M, Carboni G, Frau V, Giorgi O. Pentylenetetrazole–induced kindling in rats: Effects of GABA function inhibitors. *Pharmacol Biochem Behav* 1991; 40: 329–333.

39. Nagai T, Suzuki Y, Arai H, Imai K, Kodaka R, Itagaki Y, Tanaka J, Ono J, Okada S. Effects of pentylenetetrazole (PTZ) kindling on GABAergic system: A histochemical study by staining for GABA–transaminases (GABA–T). *Jpn J Psychiatr Neurol* 1993; 47: 392–393.

40. Remler MP, Sigvardt K, Marcussen WH. Pharmacological response of systemically derived focal epileptic lesions. *Epilepsia* 1986; 27: 671–677.

41. Porter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Seoville B, White BG. Antiepileptic drug development programme. *Clev Clin Quart* 1984; 103: 287–293.

42. Ticku MK, Rastogi SK. Convulsant/anticonvulsant drugs and GABAergic transmission. In Nistico G, Morseilli PL, Llloyd KG, Fareillo RG and Engel J Jr. (Eds.) *Neurotransmitters, Seizures and Epilepsy* Raven Press New York, 1986; pp. 163–177.

43. Cepeda C, Tanaka T, Besselievre R, Potier P, Naquet R, Rossier J. Proconvulsant effects in baboons of ß-carboline, a putative endogenous ligand for benzodiazepine receptors. *Neurosci Lett* 1981; 24: 53–57.

44. Cowen PJ, Green AR, Nutt DJ, Martin IL. Ethyl ß-carboline carboxylate lowers seizure threshold and antagonizes flurazepam-induced sedation in rats. *Nature* 1981; 290: 54–55.

45. Johnson DD, Fisher TE, Tuchek JM, Crawford RD. Pharmacology of methyl and propyl ß-carbolines in a hereditary model of epilepsy. *Neuropharmacology* 1984; 23: 1015–1017.

46. Prado de Carvallho L, Grecksch G, Cavalheiro EA, Dodd RH, Chapouthe G, Rossier J. Characterization of convulsions induced by methyl ß-carboline–3–carboxylate in mice. 1984; 103: 287–293.

47. Petersen EN. DMCM: a potent convulsive benzodiazepine receptor ligand. *Eur J Pharmacol* 1983; 94: 117–124.

48. Braestrup C, Nielsen M, Olsen CE. Urinary and brain ß-carboline–3–carboxylates as potent inhibitors of brain benzodiazepine receptors. *Proc Natl* Acad Sci *(USA)* 1980; 77: 2288–2292.

49. Snead OC. Gammahydroxybutyrate in the monkey. *Life Sci* 1978; 20: 1935–1944.

50. Depaulis A, Bourgignon J, Marescaux C, Vergnes M, Schmitt M, Micheletti G, Warter JM. Effect of r–hydroxybutyrate and r–butyrolactone derivatives on spontaneous generalized non-convulsive seizures in the rat. *Neuropharmacology* 1988; 27: 683–689.

51. Depaulis A, Snead OC, Marescaux C, Vergnes M. Suppressive effect of intranigral injection of muscimol in three models of generalized non convulsive seizures in the rat. *Brain Res* 1989; 498: 64–72.

52. Snead OC. r–hydroxybutyrate model of generalized absence seizures: further characterization and comparison with other absence models. *Epilepsia* 1988; 29: 361–368.

53. Snead III OC. The r–hydroxybutyrate model of absence seizures: correlation of regional brain levels of r–hydroxybutyric acid and r–butyrolactone with spike wave discharges. *Neuropharmacology* 1991; 30: 161–167.

54. Ono K, Baba H, Sugai S. A new chronic model of spontaneous nonconvulsive generalized seizures. *Japan J Psychiatr Neurol* 1993; 47: 396–397.

55. Hosli E, Hosli L. Binding sites for [^{3}H]G–hydroxbutyrate on cultured neurones of rat cerebellum and spinal cord. *Neurosci Lett* 1983; 42: 145–148.

56. Roth RH, Doherty JD, Walters JR. Gamma–hydroxybutyrate: a role in the regulation of central depaminergic neurons. *Brain Res* 1980; 189: 556–560.

57. Turski L, Niemann W, Stephens DN. Differential effects of antiepileptic drugs and ß carbolines on seizures induced by excitatory amino acids. *Neurosciences* 1990; 39: 799–807.

58. Olney JW, Rhee V, Ho OL Kainic acid: a powerful neurotoxic analog of glutamate. *Brain Res* 1974; 77: 501–512.

59. Scerrati M, Onofri M, Pacifici L, Pola P, Rammaci MT, Rossi GF. Electrocerebral and behavioural analysis of systemic kainic acid-induced epilepsy in the rat. *Drug Exp Clin Res* 1986; 12: 671–680.

60. Lockard JS, Uhlir V, Ducharme LL, Farquhar JA, Huntsman BJ. Efficacy of standard anticonvulsants in monkey model with spontaneous motor seizures. *Epilepsia* 1975; 16: 301–317.

61. Tanaka T, Kaijima M, Yonemasu Y, Cepeda C. Spontaneous secondarily generalized seizures induced by a single microinjection of kainic acid into unilateral amygadala in cats. *Electroenceph Clin Neurophysiol* 1985; 61: 422–429.

62. Ben-Ari Y. Limbic Seizure and brain damage produced by kainic acid: mechanisms and relevance to humantemporal lobe epilepsy. *Neuroscience* 1985; 14: 375–408.

63. Carrea R, Lanari A. Chronic effect of tetanus toxin applied locally to the cerebral cortex of dog. *Science* 1962; 137: 342–343.

64. Melanby J, Hawkins C, Mellanby H, Rawlins JNP, Impey ME. Tetanus as a tool for studying epilepsy. *J Physiol* 1984; 79: 207–215.

65. Van Heyningen S. Tetanus toxin. *Pharmacol Ther* 1980; 11: 477–484.

66. Walker AE, Johnson HC. Convulsive factor in commercial penicillin. *Arch Surg* 1945; 50: 69–73.

67. Fariello RG. Parenteral penicillin in rats: an experimental model for multifocal epilepsy. *Epilepsia* 1976; 16: 217–222.

68. Prince DA and Farell D. Centrencephalic spike wave discharges following parenteral penicillin injection in the rat. *Neurology* 1969; 19: 309–310.

69. Fisher RS and Prince DA. Spike–wave rhythm in cat cortex induced by parenteral penicillin 1. Electroencephalographic features. *Electroencephal Clin Neurophysiol* 1977; 42: 608–624.

70. Guberman A, Gloor P, Sherwin AL. Response of generalized penicillin epilepsy in the cat to ethosuximide and diphenhydantoin. *Neurology* 1975; 25: 785–864.

71. Pellegrini N, Gloor P, Sherwin AL. Effect of valproate sodium on generalized penicillin epilepsy in the cat. *Epilepsia* 1978; 19: 351–360.

72. Kopeloff LM, Chusid JG, Kopeloff N. Epilepsy in Macacca mulatta after cortical or intracerebral alumina. *Arch Neurol Psychiatry* 1955; 74: 523–526.

73. Hanna GR, Stalmaster RM. Cortical epileptic lesions produced by freezing. *Neurology* 1973; 23: 918–925.

74. Loiseau H, Averet N, Arrigoni F, Cohadon F. The early phase of cryogenic lesions: an experimental model of seizures updated. *Epilepsia* 1987; 28: 251–258.

75. Karpiak SE, Graf L, Rapport MM. Antibodies to brain gangliosides produces recurrent epileptiform activity. *Science* 1976; 194: 735–737.

76. Karpiak SE, Mahadik SP, Fraf L, Rapport MM. An immunological model of epilepsy: seizures induced by antibodies to G.M.1 ganglioside. *Epilepsia* 1981; 22: 189–196.

77. Gupta YK, Malhotra J. Influence of adenosine agonists and antiepileptic drugs on theophylline-induced seizures in rats. *Indian J Physiol Pharmacol* 1998; 42: 491–497

78. Chang CWJ, Bleck TP. Status Epilepticus. *Neurologic Clinics* 1995; 13: 529–548.

79. McIntyre DC, Edson N. Kindling based status epilepticus, effects of norepinephrine depletion with 6–hydroxydopamine. *Exp Neurol* 1989; 104: 10–14.

80. McIntyre DC, Stokes KA, Edson N. Status epilepticus following stimulation of a kindled hippocampal focus in intact and commissurotomized rats. *Exp Neurol* 1986; 94: 554–570.

81. Vicedomini JP, Nadler V. A model of status epilepticus based on electrical stimulation of hippocampal afferent pathways. *Exp Neurol* 1987; 96: 681–691.

82. Taber KH, McNamara JJ, Zornetzer SF. Status epilepticus: a new rodent model. *Electroencephal Clin Neurophysiol* 1977; 43: 707–724.

83. Lothman EW, Bertram EH, Bekenstein JW, Perlin JB. Self sustaining limbic status epilepticus induced by 'continuous' hippocampal stimulation. Electrographic and behavioural characteristics. *Epilepsy Res* 1989; 3: 107–119.

84. George B, Mathur R, Kulkarni SK. Development of self sustaining limbic status epilepticus by continuous ventral hippocampal stimulation followed by low dose pilocarpine in rats. *Indian J Physiol Pharmacol* 1998; 42.

85. Meldrum BS, Horton RW. Convulsive effects of 4–deoxypryridoxine and of bicuculline in photosensitive baboons (Papio papio) and in rhesus monkey (Macaca mulatta). *Brain Res* 1971; 35: 419–436.

86. Walton NY, Treiman DM. Experimental secondarily generalized convulsive status epilepticus induced by DL–homocysteine thiolactone. *Epilepsy Res* 1988; 2: 79–86.

87. Chronopoulos A, Strafstrom C, Thurber S, Hyde P, Mikati M, Holmes GL. Neuroprotective effect of felbamate after kainic-acid-induced status epilepticus. *Epilepsia* 1993; 34: 359–366.

88. Stafstrom C, Thompson JL, Chronopoulos A, Thurber S, Holmes GL. Effect of age on behavioural abnormalities following kainic acid-induced status epilepticus. *Ann Neurol* 1990; 28: 467–468.

89. Olney JW. Excitotoxins : an overview. In Excitotoxins. Fuxe K, Roberts P, Schwartz R (Eds.) Macmillan press, London, 1983; pp 82–96.

90. Turski WA, Cavalheiro EA, Schwarz M, Czuczwar SJ, Kleinrok Z, Turski L. Limbic seizures produced by pilocarpine in rats: Behavioural, electroencephalographic and neuropathological study. *Behav Brain Res* 1983; 9: 315–336.

91. Turski WA, Cavalheiro EA, Coimbra C, Berzaghi MP, Ikonomidou-Turski C, Turski L. Only certain antiepileptic drugs prevent seizures induced by pilocarpine. *Brain Res Rev* 1987; 12: 281–305.

92. Persinger MA, Makarec K, Bradley JC. Characteristics of limbic seizures evoked by peripheral injections of lithium and pilocarpine. *Physiol Behav* 1988; 44: 27–37.

93. George B, Mathur R, Kulkarni SK. Protective effect of acute clozapine on pilocarpine models of status epilepticus in rats: a behavioural, electroencephalographic and neuropathological study. *Asia Pac J Pharmacol* 1998; communicated.

94. Kulkarni SK, George B. Lithium-pilocarpine neurotoxicity: A potential model of status epilepticus. *Meth Find Exp Clin Pharmacol* 1995; 17: 551–567.

95. Chaudhary G, Chaudhary JD, malhotra J, Gopinath G, Gupta YK. Status epilepticus by pilocarpine in lithium primed rats : Does treatment schedule affect seizures and neuromal degeneration. *Indian J Physiol Pharmacol* 1997; 41(5suppl): 480A.

96. Yokoi I, Toma J, Liu J, Kabuto H, Mori A. Adenosines scavenged hydroxylradicals and prevented post-traumatic epilespy. *Free Rad Biol Med* 1995; 19: 473–479.

97. Kabuto H, Yokoi I, Ogawa N. Melatonin inhigits iron-induced epileptic discharges in rats by suppressing peroxidation. *Epilepsia* 1998; 39: 237–243.

98. Willmore LJ, Hurd RW, Sypert GW. Epileptiform activity initiated by pial iontophoresis on ferrous and ferric chloride on rat cerebral cortex. *Brain Res* 1978; 152: 406–410.

99. willmore LJ, Sypert GW, Munson JB. Recurrent seizures induced by cortical iron injection: A model of post-traumatic epilepsy. *Ann Neurol* 1978; 4: 329–336.

100. Willmore LJ, Sypert GW, Munson JB, Hurd RW. Chronic focal epileptiform discharges induced by injection of iron into rat and cat cortex. *Science* 1978; 200: 150–153.

101. Rosen AD, Frumin NV. Focal epileptogensis after intracortical hemoglobin injection. *Exp Neurol* 1979; 66: 277–284.

102. Gross PM, Weaver DF. A new experimental model of epilepsy based on the intraventricular injection of endothelin. *J Cardiovasc Pharmacol* 1993; 22 Suppl 8: S282–S287.

103. Backstrom T, Gee KW, Lan N, Sorensen M, Wahlstrom G. Steroids in relation to epilepsy and anesthesia. In: M.A. Simmods (Eds.), Steroids and Neuronal activity, Wiley, Chichester, 1990; pp. 225–239.

104. Tauboll E, Lundervold A, Gjerstad L. Temporal distribution of seizures in epilepsy. *Epilepsy Res* 1991; 8: 153–165.

105. Wahnschaffe U, Loscher W. Lack of chages in seizure susceptibility during the estrous cycle in kindled rats. *Epilepsy Res* 1992; 13: 199–204.

106. Bourgeois BFD, Dodson WE, Ferrendelli JA. Primidone, phenobarbital and PEMA. I. Seizure protection, neurotoxicity, and therapeutic index of individual compounds in mice. *Neurology* 1983; 33: 283–290.

107. Gladding GD, Kupferberg HJ, Swinyard EA. Antiepileptic drug develoment programme. In : H.H. Frey and D. Janz (Eds.) Antiepileptic drugs. Handbook of experimental pharmacology, Vol. 74, Springer–Berlin, 1985; pp. 341–347.

108. Jones GL, Wimbish GH. Hydantoins. In : H.H. Frey and D. Janz (Eds.) Antiepileptic drugs. Handbook of experimental pharmacology, Vol. 74, Springer–Berlin, 1985; pp. 351–419.

109. Kupferberg HJ. Antiepileptic drug development program : A cooperative effort of goverenment and Industry. *Epilepsia* 1989; 30 (suppl. 1): S51–S56.

110. Frey HH. Primidone, In : H.H. Frey and D. Janz (Eds.) Antiepileptic drugs. Handbook of experimental pharmacology, Vol. 74, Springer–Berlin, 1985; pp. 449–477.

111. Schmutz M. Carbamazepine. In : H.H. Frey and D. Janz (Eds.) Antiepileptic drugs. Handbook of experimental pharmacology, Vol. 74, Springer–Berlin, 1985; pp. 479–506.

112. Worms P, Lloyd KG. Functional alterations of GABA synapses in relation to sizures. In : P.L. Morselli, K.G. Lloyd, W. Loscher, B Meldrum and E. H. Reynolds (Eds.), Neurotransmitters, seizures and epilepsy. Raven Press, New York, 1981; pp. 37–48.

113. Loscher W. GABA–mimetics in animal models of seizure states. In : G. Bartholini, L. Bossi, K.G. Lloyd and P.L. Morselli (Eds.), Epilepsy and GABA receptor agonists, basic and therapeutic research, Raven Press, New York, 1985; pp. 109–119.

114. Chweh AY, Swinyard EA, Wolf HH, Kupferberg HJ. Effect of GABA agonists on the neurotoxicity and anticonvulsant activity of benzodiazepines. *Life Sci* 1985; 36: 737–744.

115. Paul V, Kazi M. A technique for qunatitative measurement of clonic convulsions in rats. *Indian J Physiol Pharmacol* 1994; 38: 125–128.

116. Malhotra J, Velpandian T, Gupta YK. A simple device for recording seizure activity in rats. *Meth Find Exp Clin Pharmacol* 1997; 19: 47–51.