Animal Models of Epilepsy

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1 Introduction

Epilepsy is the second most common neurological disorder with a predisposition to the occurrence of frequent seizures, affecting around 1.4–1.5 per 1000 in Asian countries and about 65 million people worldwide, and 3 million people in USA. Epileptic seizures are characterized by abnormal and excessive electrical discharge in a population of neuron due to imbalance between excitation and inhibition. It is proposed that changes in the inhibition have underpinned the development of epilepsy and predisposition to seizures in brain. γ-Amino butyric acid (GABA) a principle inhibitory neurotransmitter in central nervous system (CNS), formed within presynaptic GABAergic axon terminals and act on $GABA_A$ and $GABA_B$ receptors, hyperpolarize the neuronal cell by increasing chloride conductance and opening of potassium channels with decreasing Ca^{2+} entry, respectively. Several point mutations altering the synaptic and extrasynaptic functioning of $GABA_A$

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P.K. Bansal and R. Deshmukh (eds.), *Animal Models of Neurological Disorders*, https://doi.org/10.1007/978-981-10-5981-0_5

receptor are implicated in various types of epilepsies. Most often seizures occur at the neonatal stage and reflect the intense and long-term outcomes such as epilepsy, cognitive impairment, and motor disorder. Phenobarbitals and benzodiazepines are most effective in the treatment of epilepsy in adults but failed to produce positive results in neonates. Several co-morbidities such as depression and cognitive deficit in the epileptic patients majorly affect the overall lifestyle and healthcare cost. Antiepileptic drugs, which are clinically used to treat the epileptic seizures, can also produce certain psychiatric co-morbidities. Moreover, clinically used antiepileptic drugs also reported to produce secondary complications.

2 Need of Animal Models

In present decennary, several antiepileptic drugs (AEDs) are available but the development of new antiepileptic drugs with the more appropriate tolerability and efficacy is still a chief concern. An ideal animal model is always required to determine the safety and efficacy of newer drug, prior to be tested in human subjects, and to determine relationship between epilepsy and its related secondary complications. Animal models play important role to understand the pathophysiology and pattern of disease progression. Animal models can be used to establish safety and efficacy of novel AEDs over clinically used AEDs. However, a single animal model cannot be efficient for the all above purposes. After the establishment of anticonvulsant potential of new AEDs in a simple model like maximal electroconvulsive seizures (MES) or pentylenetetrazol (PTZ) test, different animal models like kindling model of temporal lobe can be used to investigate the anticonvulsant spectrum of novel AEDs.

2.1 Characteristics of an Ideal Animal Model of Epilepsy

An experimental animal model is designed in such a way that it should possess the following features as described in Table 1.

Table 1 Characteristics of seizure in animal model

Sr. No	Characteristics of seizure model
1.	Seizures should be spontaneous and recurrent
2.	Clinically relevant to human epileptic seizure
3.	Electroencephalography (EEG) pattern should be similar to clinical epileptic observations
4.	Intensity of seizures should be sufficient for acute and chronic dosing of drugs in experimental studies
5.	It should posses cognitive and neurobehavioural alteration
6.	Latent period should occur between brain insult and seizure

2.2 Score

(1) Seizure severity—With respect to the strength, motor seizure is classified on a five-point scale, at the beginning little or no motor activity with after discharge (AD) exploratory behaviour. Jaw opening and head nodding is the first sign of skeletal seizure, which occurs 2–4 days before the occurrence of full motor seizure, next element of motor seizure is forelimb contraction and falling, and the full motor seizure rearing and falling.

Stage 1—Mouth and facial movements
Stage 2—Head nodding
Stage 3—Forelimb clonus
Stage 4—Rearing
Stage 5—Rearing and falling

3 Classification of Seizure

Partial seizure (Focal onset)

Simple partial (Consciousness is not impaired)

- With motor signs
- With somatosensory and special sensory symptoms
- With autonomic symptoms
- With psychic symptoms

Complex partial (With impairment of consciousness)

- Starts as simple partial and lead to impairment of consciousness
- With impairment of consciousness at onset

Partial seizure to the secondary generalization

- Simple partial to the secondary generalization
- Complex partial to the secondary generalization
- Simple partial involving complex partial

Generalized seizure

Absence seizure

- Impairment of consciousness only
- With mild clonic component
- With mild atonic component
- With automatism

Myoclonic seizure
Clonic seizure
Tonic seizure
Tonic-clonic seizure
Atonic seizure

4 Pathophysiology of Seizures

An absence seizure is non-convulsive and causes brief loss of consciousness, which arises and terminates suddenly. It is most privileged type of seizure in childhood age. The EEG study of patient with absence epilepsy showed slow spike wave discharge (2–4 Hz) bilaterally. Thalamus is the major relay centre for all sensory and motor perception, and it is also involved in spread of absence seizure. Reticular thalamic nucleus (TRN) covers rostral, lateral, and ventral parts of thalamus, and spreads spike wave discharge (SWD). Reticular thalamic nucleus made up of GABAergic interneuron and thalamocortical nucleus (TC), which is primarily excitatory in nature, contains T-type Ca^{2+} channel and involved in rebound burst firing of neuron (Figs. 1, 2).

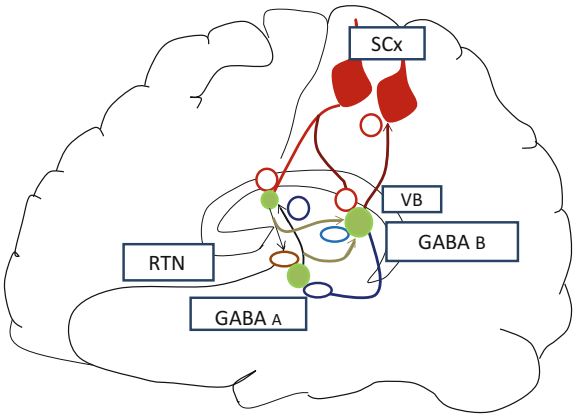

Fig. 1 Neuronal pathways involved in pathophysiology of absence seizures. Glutamatergic projection appears from somatosensory cortex (SCx), and it forms synapse with the ventrobasal posterior thalamus (VB), further forming excitatory synapse with SCx. Thalamic reticular neurons are primarily GABAergic and make coordination with these excitatory loops, by causing hyperpolarization. During the 'absence seizure', neuron of RTN became more hyperpolarized by activation of T-type Ca^{2+} channels and opening of these channels due to depolarization on VB region. RTN neuron hyperpolarizes the VB neurons by $GABA_A$ and $GABA_B$ and initiates the opening of T-type Ca^{2+} channels present on this cells; thus, rebound burst firing occurs from thalamus to SCx

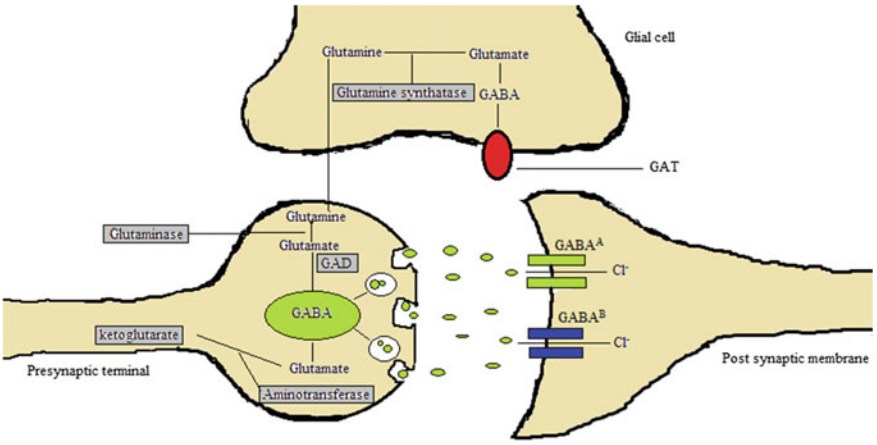

Fig. 2 GABAergic pathway in brain

5 Classification of Animal Models of Epilepsy (Fig. 3)

5.1 Chemical-Induced Convulsions

5.1.1 Pentylenetetrazol-Induced Convulsions

Principle—The convulsing action of PTZ is due to the blocking of $GABA_A$ receptor and inhibition of the chloride channels opening. Usually, it binds to the picrotoxin binding site on $GABA_A$ receptor. Single dose of PTZ in rats significantly decreases the mRNA level of $GABA_A$ receptors and its surface availability. Single pentylenetetrazol dose in albino mice causes myoclonic, clonic than generalized tonic-clonic convulsions (Fig. 4).

Acute treatment: PTZ-induced convulsion is validated model of generalized absence, myoclonic seizure, and clonic convulsion, due to the cortical stimulation. Different dose of PTZ in different species is despite below.

Sr. No	Species	Dose (single)	Reference
1	Male Swiss mice	95 mg/kg s.c	Kaminski et al. (2001)
2	Wistar rat	60 mg/kg i.p	Malhotra and Gupta (1997)
3	Male SD rat	45 mg/kg i.p	Walsh et al. (1999)
4	Male NMRI mice	60 mg/kg i.p	Ahmadini et al. (2003)
5	Swiss mice	50 mg/kg i.p	Medina et al. (2001)

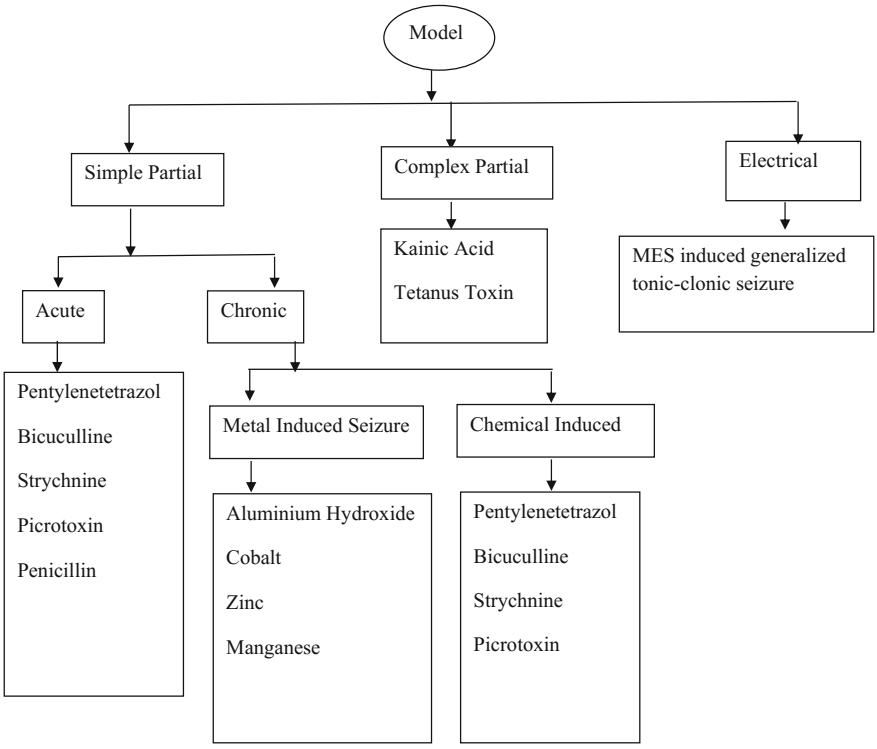

Fig. 3 Classification of animal models of epilepsy

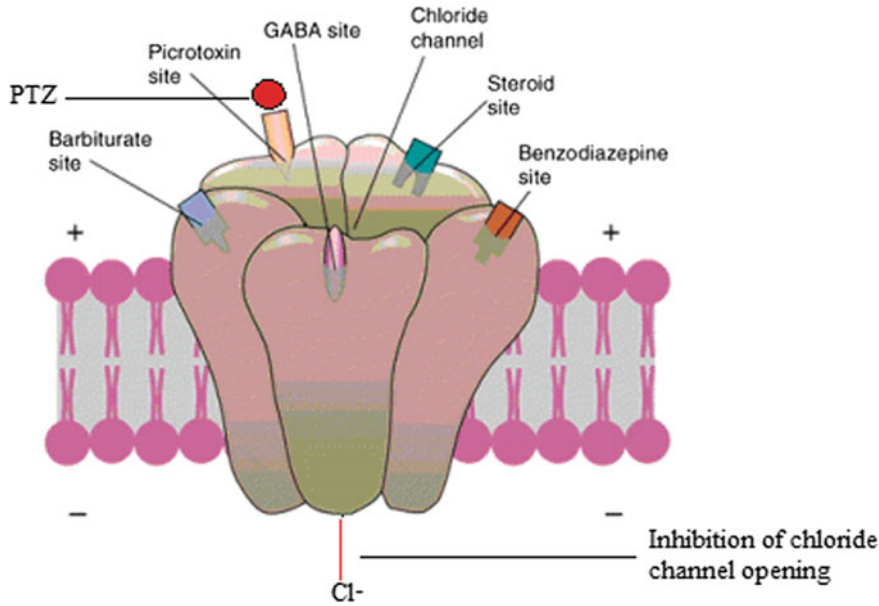

Fig. 4 Pentylenetetrazol (PTZ)-induced convulsions

Chronic treatment: Goddard in 1967 described that kindling is a validated model of epilepsy which causes generalized seizure. Repeated administration of sub-convulsive dose of chemoconvulsant like PTZ causes tonic-clonic seizure, and at this stage, animal is thought to be fully kindled.

Sr. No	Species	PTZ dose	No. of injection	References
1	Male Swiss albino mice	35 mg/kg i.p	11 (on alternate days)	Ilhan et al. (2005)
2	Male Wistar rat	30 mg/kg i.p	13 (on alternate days)	Atack et al. (2000)
3	Male Wistar rat	30 mg/kg i.p	21 (on alternate days)	Atapour et al. (2000)
4	Male Wistar rat	45 mg/kg	13 (on alternate days)	Phole et al. (1997)

Advantage—PTZ administration produces myoclonic jerk followed by the generalized tonic-clonic seizures. In the process of kindling, there is increase in seizure severity with reduction of seizure threshold as well as neurodegeneration in limbic region is found similar to human mesotemporal epilepsy. This pattern has also been observed in the electrical amygdala kindling.

Disadvantage—Phenytoin, carbamazepine, oxcarbazepine are not effective against pentylenetetrazol-induced seizures while ethosuximide, benzodiazepines, vigabatrin, tiagabine are effective in this model.

5.1.2 Bicuculline-Induced Convulsions

Principle—Bicuculline acts on $GABA_A$ receptor in a competitive manner and inhibits the chloride channel opening. Bicuculline causes spike wave discharge (SWD) after focal and systemic application and tonic-clonic seizure activity.

Acute treatment:

Sr. No	Species	Weight	Dose (single)	Reference
1	Male Laca albino mice	22–30 gm	4 mg/kg i.p.	Dhir et al. (2006)
2	Male SD rat	150–200 gm	0.375 mg/kg i.v.	Bowdler et al. (1980)

Chronic treatment:

Bicuculline Methionide Kindling Chemitrode, stainless steel guide cannulae (0.60 mm in o.d and 0.32 mm in i.d), which contain bipolar stainless steel electrode on its external wall and Mandrell (0.29 mm in diameter) were implanted in left Basolateral Amygdala. Stainless steel Mandrell (0.29 mm in diameter) then removed and replaced by the stainless steel injection cannulae (0.29 mm in o.d).

After 2 weeks of implantation, implanted animals were injected Bicuculline methionide injection (0.2 n mole dissolved in 0.8 µl sterile water containing NaCl with pH 6.2) once every fourth day. Injection was delivered over 30 s by using Hamilton syringe. Epileptic seizure is produced 10 min after injection. Additional 0.2 n mole Bicuculline methionide was injected if first injection failed to produce response.

Assessment of Seizure

Stage 0—No symptoms
Stage 1—Mouth and facial movement
Stage 2—Head nodding
Stage 3—Forelimb clonus
Stage 4—Rearing
Stage 5—Rearing and falling

Advantage—Bicuculline methionide induces chronic and abnormal functional changes instead of structural lesion. Bicuculline methionide-induced seizure lasts for at least 6 months and similar to electrical amygdala kindling.

Disadvantage—Many clinically available anticonvulsant drugs like diazepam are not effective towards the Bicuculline-induced seizure, but carbamazepine and phenytoin are effective.

5.1.3 Strychnine-Induced Convulsions

Principle—Glycine is the inhibitory neurotransmitter, which controls the motor rhythm generation in the brain stem and spinal cord. Glycine acts by strychnine-sensitive glycine receptor (GlyRs), which is highly found in medulla oblongata, spinal cord, and brain stem (Betz and Laube 2006). Strychnine is competitive antagonist of glycine receptor, and by antagonizing the inhibitory effect of glycine on brain stem and spinal cord, it causes convulsions.

Acute treatment:

Sr. No	Species	Dose (single)	References
1	Male mice	2 mg/kg s.c	Bogdanova et al. (1997)
2	Male albino mice	4 mg/kg i.m	Olatokunboh et al. (2009)

Chronic treatment—Strychnine is given to the Wistar albino rat at the dose of 15 mg/kg i.p, or 30 mg/kg i.p on alternate days for 15 days. Seizure are evaluated as

Score 0 = no seizure
Score 1 = jerks
Score 2 = straub tail
Score 3 = clonic convulsions

Advantage—Similar pattern of generalized tonic-clonic convulsions can be produced by the strychnine.

Disadvantage—Strychnine-induced seizure pattern is different from the seizures produced by primary GABA antagonist.

5.1.4 Picrotoxin-Induced Convulsions

Principle—Picrotoxin is a $GABA_A$ receptor antagonist. It binds to the β_2/β_3 subunit of $GABA_A$ receptor and blocks the opening of chlorine channel and causes intense tonic-clonic seizure.

Procedure (Acute treatment)

Sr. No	Species	Dose (single)	Reference
1	Laka albino mice	4 or 8 mg/kg i.p	Akula et al.,2007
2	Male Wistar rat	5 mg/kg i.p	Paul and Subramanian (2002)
3	Wistar rat	3 mg/kg i.p	Paul and Krishnamoorthy (1998)

Chronic treatment—Male Wistar rats (200–320 gm) were treated with sub-convulsive dose of picrotoxin (1.5 mg/kg i.p) 20 injections per day for 2–3 days. Stages 3–4 (hindlimb clonus with rearing as well as generalized tonic clonic with rearing and falling) were observed.

Advantage—Many drugs are used to produce partial seizure by focal application, but picrotoxin produces tonic-clonic seizure by the systemic application. It also causes burst firing of dopaminergic neuron.

Disadvantage—Valproate and ethosuximide have very low action towards the pilocarpine-induced convulsions compared to the diazepam, carbamazepine, and phenytoin. Small neurotoxic dose of valproate is required to attenuate the convulsions.

5.1.5 Kainic Acid-Induced Convulsions

Principle—Kainic acid (KA) is analogue of glutamate, which is primarily excitatory neurotransmitter and agonist of AMPA/kainate class of glutamate receptor. Kainic acid-induced limbic seizures are validated models, which on systemic or intracerebral administration produces limbic seizure. Hippocampus is more vulnerable for kainic acid-induced injury. Study revealed that administration of kainic acid decreased the size of hippocampus and increased the size of ventricles. Kainic acid produces complex partial seizures, leading to the secondary generalization in a dose-dependent manner. The first symptom of kainic acid administration is automatism, which is known as ‘wet dog shakes’, and at the sufficient dose,

this automatism is followed by motor seizure for several hours. Thus, KA injections represent the model of convulsive status epilepticus.

Treatment:

Sr. No	Species	Dose (single)	Reference
1.	Wistar rat	10 mg/kg i.p	Gupta et al. (2006)
2.	Albino Wistar rat	6 mg/kg i.p	Konrad et al. (1999)

Advantage—Less mortality rate can be achieved by direct application of kainic acid in brain compared to its systemic application. It is best animal model for mesial temporal lobe epilepsy (MTLE).

Disadvantage—Kainic acid sensitivity varies from different mouse strains, whereas C57BL/6, C57BL/10, and F1 C57BL/6 strains are resistant to systemic administration.

5.1.6 Tetanus Toxin-Induced Convulsions

Principle—Synaptobrevin is a protein and required for the neurotransmitter release. Tetanus toxin causes breakdown of this protein and inhibits the release of inhibitory neurotransmitter, which leads to chronic epileptic stage. Little mossy fibre sprouting also occurs in hippocampus with tetanus toxin administration. Study revealed that single micro-injection of tetanus toxin produces the model of complex partial seizures clinically similar to the human complex partial seizures, and these seizures occur for at least 3 min spontaneously and occasionally for several weeks or months.

Dorsal Hippocampal Administration of Tetanus Toxin (TT)

Animal	Anaesthesia	Craniotomy	Co-ordinates/dose	Reference
Male SD rats	1.5–3.0% inhaled isoflurane with 0.05 mg/kg buprenorphine	Over right hippocampus (3.5 mm posterior and 2.8 mm lateral to bregma)	25 ng of TT in 0.5 μl phosphate-buffered saline with 0.2% bovine serum albumin is administered at 3.3 mm posterior to bregma, 3.2 mm lateral, and 3.1 mm ventral	Rolston et al. (2010)

Advantage—Cortical application of tetanus toxin in cats produces Epilepsia Partialis Continua (EPC) syndrome similar to humans. It causes long-lasting changes in the synaptic excitation with no/minimal neuronal damage. Seizure induced by tetanus toxin continues for at least 1–3 weeks and thus offers long-term treatment studies.

Disadvantage—Tetanus toxin administration in brain is used to produce the model of MTLE. However, seizures induced by the tetanus toxin is week or short-term. Therefore, this model has low acceptance compared to the kindling and post-status epilepticus model.

5.1.7 Penicillin-Induced Convulsions

Principle—Penicillin produces experimental model of partial seizure by selectively antagonizing the inhibitory effect of $GABA_A$ receptor. In consideration to clinical data, systemic administration of penicillin at high dose produces myoclonic and tonic-clonic seizures in human.

Treatment:

Animal	Anaesthesia	Co-ordinates	Dose
Male Wistar rat	Ketamine hydrochloride 100 mg/kg i.p	Cannulae were implanted to right lateral cerebral ventricle (0.6 mm posterior to bregma, 2.0 mm lateral to midline, 4.2 mm below the surface of scull)	Penicillin G potassium 300 IU, in 3 µl.

Behavioural Observations

Score 0—No response
Score 1—Twitching of face and ear, fictive scratching
Score 2—Myoclonic twitching and tremor
Score 3—Bilateral forelimb clonus
Score 4—Tonic-clonic seizure
Score 5—Tonic-clonic seizure without retention of postural control (Bostamci and Bagirici, 2007)

Advantage—It is appropriate model to investigate the spread of seizure activity. Penicillin-induced epileptic seizure starts locally, but latter it spreads and converts to generalized epilepsy, thus resembling the model of grand mal epilepsy.

(H) Metal-induced convulsions

(i) Aluminium hydroxide

Principle—This is the validated model of temporal lobe epilepsy. Aluminium hydroxide leads to partial seizures (rhythmic jerking) followed by secondary generalized tonic-clonic seizure. Similar pattern can be seen in cat and monkey as in humans, such as gliosis, cell loss, and neovascularization at the implantation site of aluminium.

Procedure

(1) Animal—Adult male cats

Anaesthesia—Suitable anaesthesia with adjuvants

Aluminium hydroxide administration—Cannulae made up of 22.0 g spinal quincke needles were implanted to the anterior sigmoid gyrus (anterior +27 mm, lateral 3.5 mm, and ventral 1.0 mm below the dural surface) and in the basolateral amygdala (anterior +1.20 mm, lateral 10.0 mm, ventral −7.0 mm). 0.1 ml sterile aluminium hydroxide was injected.

EEG recording—Insulated bipolar electrode measuring 1.0 mm at the tip was placed at the right and left posterior sigmoid gyrus (anterior +23.0, lateral 5.0), right and left anterior suprasylvian gyrus (anterior +16.0, lateral 10.0), right caudate nucleus (anterior +15.0, lateral 6.0, ventral +6.07), left and right rostra1 thalamus (anterior +13.0, lateral 2.0, ventral −3.9), and right mesencephalic reticular formation (anterior +2.0, lateral 2.0, ventral −2.5) (Feeney et al. 1998).

(2) Animal—Male rhesus monkey

Anaesthesia—Pentobarbital 35 mg/kg i.p., 4 mg/kg i.m. used to reduce oedema, atropine sulphate 0.05 mg/kg s.c. used to reduce the salivation.

Aluminium hydroxide administration—Cannulae are implanted on the temporal lobe in the following co-ordinates:

(1) Anterior hippocampus—Anterior 9.0 mm, lateral 10.1 mm, horizontal −4.0 mm

(2) Middle hippocampus—Anterior 6.0 mm, lateral 10.1 mm, horizontal −9.0 mm

(3) Posterior hippocampus—Anterior 3.0 mm, lateral 10.7 mm, horizontal 0.2 mm

Aluminium hydroxide injection was administered in volume between 0.1 and 0.3 ml.

EEG recording—Stainless steel bipolar depth electrode was used to record the EEG. It is placed over anterior and posterior hippocampus bilaterally adjacent to the aluminium injection site. EEG recording was done by using 6.4-mm-diameter, no. 4 self-tapping stainless steel screw.

Advantage—Important neuronal loss of dendritic cell in the epileptic locus can be achieved by this model. Neuronal loss decreased GABAergic neuron as well as decreased the positive terminal of GAD (glutamate decarboxylase) in the epileptic focus.

Disadvantage—This model requires six weeks to three months to get developed.

(ii) Cobalt

Principle—It is a chronic model of epilepsy. Implantation of cobalt in the cortex of animal produces primary and secondary epileptogenic locus that continues for several weeks. Study showed that tonic-clonic jerks produced by cobalt implantation might be due to increase in the glycogen concentration in the sensory-motor cortex. Study also revealed that injury made by cobalt in cortex decreased the GABAergic immunoreactive cells.

Procedure—Cobalt metal disc (diameter 3.2 mm and thickness 0.003 mm) was implanted on the sensory-motor cortex of male Wistar rat (200–300 gm) under suitable anaesthesia.

Electrocorticograph (ECoG) recording—Four stainless steel screws can be placed over sensory-motor cortex.

Advantage—Bonvall et al. developed this model. Cobalt produces chronic epileptic seizure by inhibiting the synthesis of GABA and GAD. Around 20 days of cobalt treatment, there is neuronal loss in CA1 region of hippocampus.

Disadvantage—Cobalt-induced epileptic seizures are not long-lasting, which affect the long-term epileptological study.

6 Model of Status Epilepticus

6.1 Lithium-Pilocarpine Model of Status Epilepticus

Principle—Status epilepticus (SE) may be defined as the neurological state in which repeated generalized convulsions occur lasting for 30 min which further causes neuronal injury in brain, and it is well-established model of SE related to temporal lobe. Pilocarpine-induced SE occurs within 50–60 min after injection and stage of SE is continued to the 8–12 h then it is aggravated into the recurrent seizure till the 14–25 days. This phase continued with the latent phase that leads to neurological damage followed by spontaneous and recurrent in animal for long time. Similarly in lithium-pilocarpine model, neuronal damage occurs in hippocampus, piriform cortex, entorhinal cortex, amygdala, thalamus, and septum. Among all of the structures, piriformcortex is more sensitive to the damage during SE and involved in focus of epileptic activity causing secondary generalization.

Treatment:

Animal	Dose of lithium and pilocarpine
Sprague Dawley rats	Lithium is injected at a dose of 3 mEq/kg i.p and after 24 h pilocarpine at a dose of 30 mg/kg i.p are administered (Han et al., 2009)
Albino rats	Lithium chloride 3 nmol/kg i.p is administered 24 h before pilocarpine 40 mg/kg i.p

Advantage—In this model, lithium is given to increase the effect of pilocarpine. This also reduces the dose of pilocarpine required to produce SE and induces more stable convulsions in rats.

Disadvantage—Benzodiazepines are effective against the lithium-pilocarpine-induced seizures if given earlier because seizure becomes resistant with time.

6.2 Perforant Path Stimulation Model of Status Epilepticus

Principle—Perforant path stimulation induces damage to the hippocampal neuron particularly in the CA1, CA2 region of hippocampus and dentate hilus in rodent, which is similar to clinical SE conditions. Electrical stimulation also leads to mossy fibre sprouting in hillar region of dentate gyrus.

Procedure

Animal—Male Sprague Dawley rat (250–320 gm)

Anaesthesia—Suitable

Electrode implantation—Twisted 125-μm Teflon-coated stainless steel wire is implanted 4.4 mm lateral, 8.0 mm caudal from bregma to stimulate the perforant path.

Stimulation—2–3 mA, 50 μs alternating monopolar pulses at 20 Hz for 2 h were used to stimulate perforant path. Behaviour is recorded by Racine classification.

Advantage—Necrosis can be induced by the perforant path stimulation model in the region of CA1, CA3, and hillar neurons. Moreover, hippocampal status epilepticus can be induced without any intervention of excitotoxic damage, which is caused by kainic acid or pilocarpine model.

Disadvantage—Perforant path stimulation causes limited damage to the dorsal hippocampus. This model shows difference in brain lesion, according to the site of stimulation and the intensity of stimulation.

7 Maximal Electroshock (MES)-Induced Convulsions

Principle—Animals can be used to study the different types of epileptic seizures such as grand mal and petitmal. Grand mal type of epilepsy can be induced in mice or rat by the maximal electroshock, in which corneal electrodes are used to induce cortical stimulation, and the seizure consequences such as tonic flexion, tonic extensor, clonic convulsion, stupor, recovery, or death can be studied in the animals.

Procedure—Tetracaine in a concentration of 0.5% in saline is applied on the eyes before the experiment and then 50 mA, 60 Hz, 0.2 s current in mice and 150 mA, 60 Hz, 0.2 s current in rat were applied through corneal electrode. The entire animals were subjected to the 6 Hz/MES test. The current intensity of 32 or 44 mA at 6 Hz for 3 s was applied by corneal electrode to check the effect of drug.

Advantage—MES induces single generalized tonic-clonic convulsion in animals which can be seen in drug-resistant epileptic patient.

Disadvantage—This model is not effective for the anticonvulsant drugs which increase the seizure threshold but not have enough power to increase the threshold above 50 mA for mice and 150 mA for rats.

Acquired Epilepsy (Chronic model) Pathogenesis of acquired epilepsy (AE) is thought to be due to brain insult, which causes abnormal remodelling of neuron or neuronal network. At the time of brain insult, there is elevation of [Ca^{2+}] overload. If the injury or insult is very intense, it causes neuronal death. Development of AE occurs in different stages like injury to the brain followed by epiliptogenesis and spontaneous recurrent seizures. The ratio of acquired epileptic patients is about 50%, and rest of these are idiopathic.

8 Animal Models of Temporal Lobe Epilepsy

The animal model of TLE is widely used to understand the underlying basic mechanism of epiliptogenesis, and it resembles the similar characteristics as human epilepsy like mossy fibre sprouting and neuronal loss. It is also beneficial to know the basic structural and neuronal changes, causes interictal behavioural disturbance, and is often associated with TLE. Epileptic seizure originated from temporal lobe is always manifested with aura (an unusual sensation) like epigastric discomfort, hallucination, sensory and motor perceptions. Psychiatric problems such as depression, anxiety are the most common interictal emotional disturbance in TLE patients compared with schizophrenic patients (Klynchuk et al. 2000).

8.1 Amygdala Kindling

Principle—Amygdala is made up of 10 nuclei, among all the nuclei; basolateral amygdala (BLA) plays a crucial role for the development and spreading of epileptic seizure. Excessive glutamatergic activity is the key component of hyperexcitability and epiliptogenesis. In the chemical and electrical kindling, overactivation of glutamatergic NMDA and AMPA receptor due to excessive glutamatergic activity leads to intense calcium influx from intracellular store, and via voltage-gated calcium channels. This leads to persistent depolarization, epiliptogenesis, and spike wave discharge (SWD). Somatostatin-immunoreactive interneuron (GABAergic interneurons of BLA) is more venerable for damage during kindling and seizure activity. Reduced GABAergic inhibition due to interneuronal loss may lead to imbalance between excitation and inhibition, resulting in epiliptogenesis in amygdala (Aroniadou-Anderjaska et al. 2008).

Right amygdala kindling

Species	Anaesthesia	Co-ordinates	EEG recording	Frequency
Male Wistar rat	Pentobarbital 50 mg/kg i.p	Bipolar electrode is implanted, anterior posterior 1.5 mm, lateral 4.4 mm, ventral 8.5 mm from bregma	From site of electrode implantation	1 s train of 50 Hz, 1 ms biphasic square wave pulse, with 500 μA amplitude is delivered until 10 fully kindled Stage 5 is not obtained

Seizure assessment—According to the Racine scale

Score 0—No seizure response
Score 1—Immobility, eye closure, ear twitching, snuffing, and facial clonus
Score 2—Head nodding
Score 3—Forelimb clonus
Score 3.5—Bilateral forelimb clonus without rearing
Score 4—Bilateral forelimb clonus with rearing
Score 4.5—Falling on a side, loss of rightning reflex accompanied by generalized clonic seizure
Score 5—Rearing and falling (Borowicz et al. 2002).

Advantage—Amygdala kindling may replicate the aspects of clinical cardiovascular complications associated with epilepsy such as hypertension and bradycardia, and it produces the similar epileptic zone which is also seen in temporal lobe epilepsy.

8.2 Hippocampal Kindling

Principle—Study reveals that kindling causes irreversible structural changes in the morphology of neuron in various brain regions, such as sprouting of mossy fibres in the granular cell of dentate gyrus in hippocampus. Sprouting may be defined as the neuronal remodelling in the dentate gyrus, in which mossy fibres grow abnormally. Entorhinal cortex (EC) gives projections to the granular cell of dentate gyrus (DG). The mossy fibres, which originate from the dentate gyrus, forward these projections to CA3 region (made up of small pyramidal cell), and Schaffer collaterals complete this circuit at CA1 region (made up of broad pyramidal cell). There is permanent loss of hillar mossy cells of DG region which provide excitatory input to the inhibitory (GABAergic) interneuron and maintain the excitation and inhibition.

Procedure Ventral hippocampal kindling

Species	Male SD rat (260–300 gm)
Anaesthesia	Chloral hydrate (400 mg/kg)
Electrode	Twisted teflon-coated stainless steel wires (diameter 0.13 mm)
EEG recording	From the tip which is not insulated
Co-ordinates	−5.4 anterior posterior from bregma, −5.2 mm lateral, −6.5 mm ventral
Frequency	To determine after discharge threshold, administer 50 μA, 60 Hz, 2 s, monophasic square waves at 1 ms/pulse. If no after discharge is found, then stimulus can be increased by 20 μA per stimulus until Stage 5 is not reached. Then, procedure is continued by 12 kindling per day at 30-min interval for 4 consecutive days with the intensity of 450 μA, 60 Hz for 10 s at 1 ms square wave pulses
Reference	Ding et al. (2010)

Advantage—Hippocampal kindling resembles the features with the patients who suffer from temporal lobe epilepsy, and it prevents memory loss associated with resective surgery.

8.3 Corneal Kindling

Principle—Corneal kindling is the easier model among all the animal models of electrical kindling. It can be used to predict the behaviour and cognitive impairment caused by the drugs like NMDA receptor antagonist and investigational new antiepileptic drug (AED). But mortality rate is relatively high compared to the classical kindling models.

Treatment:

(a) Mice—Stimulated by 3 mA, 60 Hz, 2 s once daily

(b) Rate—Stimulated by 8 mA, 60 Hz, 4 s twice daily

Stimulation is applied until Stage five on the Racine scale is not found (Kupferberg 2001)

Species	Electrode	Frequency
Male and female NMRI mice	Corneally placed saline-soaked cooper electrode	3 mA, for 3 s, 50 Hz twice daily with 6-h interval for 12 days

Seizure assessment

Score 1—Mild facial clonus and eye blinking
Score 2—Sever facial clonus, head nodding, chewing
Score 3—Unilateral or alternating forelimb clonus
Score 4—Bilateral forelimb clonus and rearing
Score 5—Bilateral forelimb clonus with rearing and falling
Score 6—Tonic hindlimb extension

Advantage—A large number of animal can be kindled within short duration. The test compound required in less amount. That is cost-effective (Kupferberg 2001).

8.4 Piriform Cortex Kindling

Principle—Piriform cortex has direct connection with the olfactory bulb, and it is highly sensitive to generate limbic motor seizure. The piriform cortex is made up of anterior and posterior cortex, and generation of seizure might be due to reduced GABAergic inhibition in the anterior piriform cortex compared to posterior. Piriform cortex is made up of three layers. Primary layer is made up of GABAergic neurons, afferent tract that originated from the olfactory bulb, and afferent fibres that originated from other neurons of piriform cortex. Secondary and tertiary layers are comprised of pyramidal cells and cell bodies, respectively.

Treatment:

Species	Anaesthesia	Electrode	Co-ordinates	Frequency	Reference
Male Wistar rat	Mixture of ketamine (100 mg/kg i.p) and diazepam (8 mg/kg i.p)	Twisted pair of 0.2-mm-diameter Teflon-coated stainless steel wires, whose tip was exposed by 0.2 mm	0.8 mm posterior from bregma, 4.9 mm left, 8.8 mm ventral	To check the after discharge threshold, 1 s train of 50 Hz square wave, for 1 ms, separated by 1-min interval. Current intensity is 7 μA and increases gradually by 20% per step to 500 μA or until a behavioural seizure occurred.	Gallego et al. (2010)

Advantage—It resembles the simple model compared to the neocortex which is six-layered and requires less stimulation compared to the hippocampal and amygdaloidal stimulation.

Disadvantage—Kindling animal model of temporal lobe epilepsy is labour intensive and requires much stimulation to elicit spontaneous seizures. Electrode implantation and chemical vaccination to the brain are little complex procedure. The lesions caused by kindling in the brain are not quite similar to the clinical observations of mesial temporal lobe epilepsy.

Ethical Statement All institutional guidelines, national guidelines, state and local laws, and regulations with professional standards for the care and use of laboratory animals should be followed. Studies involving animals must state that the institutional animal ethical committee has approved the protocol. For authors using experimental animals, a statement should be made that the animals' care is in accordance with institutional guidelines and animals used have been treated humanely and with regard for the alleviation of suffering. Researchers should treat animals as sentient and must consider their proper care and use and the avoidance or minimization of discomfort, distress, or pain as imperatives. Animal experiments should be designed only after due consideration of animal health. It should be ensured that all researchers who are using animals have received instruction in research methods and in the care, maintenance, and handling of the species being used. All the surgical procedures should be performed under appropriate anaesthesia and follow only those procedures which avoid infection and minimize pain during and after surgery.

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