



Review Article

A REVIEW: ANIMAL MODELS USED IN THE SCREENING OF ANTIEPILEPTIC DRUGS

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Abstract: Animal models for seizures and epilepsy have played a fundamental role in advancing our understanding of basic mechanisms underlying epileptogenesis and have instrumental in the discovery and preclinical development of novel antiepileptic drugs. This paper reviews the *in vivo* models like the electrically induced convulsion (e.g. maximal electroshock, kindling) and chemoconvulsants (e.g. strychnine, pentylenetetrazole, picrotoxin, isoniazid, lithium pilocarpine, yohimbine, bicuculline, 4-aminopyridine and penicillin), *in vitro* and genetic model for seizures and epilepsy.

Keywords: epilepsy, animal model, convulsion, antiepileptic drugs.

INTRODUCTION:

The development of various new antiepileptic drugs (AEDs) in recent decades, the search for new therapies with better efficacy and tolerability remains an important goal.^[1] The discovery and development of a new AED relies heavily on the preclinical use of animal models to establish efficacy and safety prior to first trials in humans.^[2] A large number and variety of animal models have been created, involving pharmacologic (e.g., pilocarpine, kainate), electrical (e.g., kindling), genetic (e.g., knock-out mice), and other injurious (e.g., trauma, hypoxia, stroke) methods or stimuli, to match the equally numerous types and causes of epilepsy in people.

In vivo models of epilepsy, in which animals exhibit actual behavioral and electroencephalographic seizures, most closely mimic the clinical features of human epilepsy. However, reduced biological systems, including brain slices, cell culture, and molecular assays, may also be advantageous in offering unique mechanistic insights into epilepsy.

In vitro models of epilepsy that allow more detailed investigations of cellular and molecular mechanisms of epileptogenesis while still preserving the critical network phenotypic features of epilepsy, particularly the development of spontaneous seizures. Intact hippocampal preparations or acute brain slices maintain much of the needed circuitry to generate electrographic seizures.^[3,4]

I. ELECTRICALLY INDUCED SEIZURES:

Maximal electroshock seizures (MES) test:

Merritt and Putnam (1938) developed the MES test and discovered the convulsive effect of diphenylhydantoin using this test.^[5] In the MES test, tonic-clonic seizures are induced by transcorneal or transauricular application of short (0.2 s) suprathreshold electrical stimulus in normal mice (50 mA) or rats (150 mA). The resultant seizures

passes through various phase: phase of tonic limb flexion of about 1.5 sec duration followed by phase of tonic limb extension lasting about 10 sec and finally followed by a variable short clonic interval which may lead to asphyxia death in some animals.^[6]

Suppression of tonic hind limb extension is taken as a measure the efficacy in this test. Drug effective against generalised tonic-clonic seizures such as phenytoin, carbamazepine, phenobarbitone and primidone. The MES test effective for generalised tonic – clonic seizures. In addition, it was proposed that the MES test may predict with efficacy against partial seizures,^[7] but the lack of anti-MES efficacy of several novel AEDs (e.g., levitracetam, tiagine, vigabatrin) that subsequently were shown to suppress partial seizures in epilepsy patients.

Kindling animal model:

Kindling is a model of epilepsy produced by repeated administration of an initially subconvulsive electrical or chemical stimulus that results in an increase in seizure activity, culminating in a generalized seizure.^[8] In rats the electrode is implanted in the right amygdale for electrical stimulation. Animal is allowed to recover from surgery for a minimum of 1-2 weeks; otherwise the sensitivity of the animals to kindling is lowered. Then daily electrical stimulus trains are applied via the electrode using either a fixed current strength (400-500 μ A, 1 msec monophasic square wave pulses for 1 sec with 50 or 60/sec frequency) or using the individual threshold current to induce after discharge at the site of stimulation.^[9]

During the daily electrical stimulation of amygdala, seizures develop into five stages:

- Class-1: Immobility, eye closure, twitching of vibrissae, stereotypic sniffing
- Class-2: Facial clonus and head nodding

Class-3: Facial clonus, head nodding and forelimb clonus

Class-4: Rearing, often accompanied by bilateral forelimb clonus

Class-5: Rearing with loss of balance and falling accompanied by generalized clonic seizures.

Rats are said to be fully kindled when enhanced sensitivity, as evidenced by class five seizures has developed.^[9] If the stimulation is continued for a few weeks, rats develop 'spontaneous' epileptic seizures that persists for as long as 7 months following termination of the stimulation.

Lothman et al described an alternate method for producing the fully kindled state in rats: the rapidly recurring hippocampal seizure (RRHS) model. The RRHS model stimulates the hippocampus of rats with 10-strains of suprathreshold tetanic electrical stimuli every few minutes. Seizure intensity was quantified using the duration of after discharge and the accompanying behavioural responses. Severe limbic seizures were elicited on the first day after the initial repeated stimulation of the hippocampus. Seizures intensified on the second day and remained stable thereafter.

Gupta YK et al^[10] described an alternative method for Kindling; a subconvulsive dose of PTZ (35 mg/kg body weight) was injected intraperitoneally on every second day for (43 days as 22 injections). The PTZ injections were stopped when the animals showed adequate kindling.

II. CHEMICALLY INDUCED CONVULSIONS:

Pentylenetetrazole (PTZ) test:

PTZ is a tetrazole derivative with consistent effect in a large number of animal species like mice, rats, cats, primates etc. It is believed to act by antagonizing the inhibitory GABAergic neurotransmission. PTZ test is used for screening of drugs affective in petit mal epilepsy or absence seizures.

In the s.c. PTZ (or metrazol) seizure test, the convulsive dose of PTZ inducing a clonic seizure of at least 5 s duration in 97% of the animals (CD97) is subcutaneously injected and animals are observed for a post-injection period of usually 30 min for the occurrence of such a "threshold" seizure.^[11] The test is thought to be predictive of anticonvulsant drug activity against nonconvulsive (absence or myoclonic) seizures. Drug effective in petit mal epilepsy like ethosuximide, vaproic acid are effective while phenytoin, carbamazepine are not effective in the PTZ model.

Strychnine model:

Strychnine-sensitive postsynaptic inhibition in higher centers of the CNS is also mediated by glycine. Strychnine induced seizures are different from those produced by primary GABA antagonists since they are mainly extensor tonic, with little cortical EEG activity^[12]. These seizures are not fully relieved by acceptable doses of any of the classical anticonvulsants including benzodiazepines.

Yamashita et al (2004) evaluated the strychnine at a dose 0.8 mg/kg was injected subcutaneous 60 min after the oral administration of test compounds. The animals were observed for 30 min after injection and wild running, clonic seizures, tonic seizures and respiratory arrest were monitored. ED₅₀ values and 95% confidence interval of tonic extension seizures were calculated^[13].

Picrotoxin model:

Picrotoxin acts as a GABA_A-antagonist modifying the function of the chloride ion channel of the GABA_A receptor complex^[14]. Picrotoxin induces minimal and maximal seizures in a dose - dependent manner. In rat, doses of 8 mg/kg produce hyperactivity, body tremors and forelimb clonus followed by tonic extension of the hind limbs and generalized tonic-clonic seizures.^[12]

Kasture et al (2002) described in mice picrotoxin (3mg/kg, s.c) was administered 30 min before the test drug. The parameter such as presence or absence of clonic convulsions was studied.^[12] The protective effect of classical anticonvulsant against picrotoxin- induced seizures has been studied while diazepam, carbamazepine and phenytoin have a protective efficacy.

Isoniazid (INH) model:

Isoniazid can precipitate convulsions in patients with seizure disorders. The compound is regarded as a GABA-synthesis inhibitor. Clonic tonic seizures are elicited in mice which are antagonized by anxiolytic drugs. In mice were treated with the test compound or the standard (e.g. diazepam 10 mg/kg i.p.) by oral or intra peritoneal administration. 30 min after i.p. or 60 min after p.o. treatment the animals was injected with a subcutaneous dose of 300 mg/kg isoniazid (INH). During the next 120 min the occurrence of clonic seizures, tonic seizures and death were recorded.^[14]

Lithium pilocarpine model:

Champerisingh D *et al* (2011) evaluated the Status epilepticus was induced by administration of pilocarpine at a dose of 350 mg/kg, i.p. Atropine 1 mg/kg i.p. was administered 30 min prior to pilocarpine to reduce the peripheral cholinergic effects of pilocarpine. Diazepam (5 mg/kg) was used as standard. The test drug was given orally 1 h before injection of pilocarpine nitrate.^[15] The severity of status epilepticus was observed every 15 min till 90 min and thereafter every 30 min till 180 min using the following scoring system such as Stage 0 - no response, Stage 1- fictive scratching, Stage 2- tremor, Stage 3-head nodding, Stage 4- Forelimb clonus and Stage 5-Rearing and falling back.^[16]

Martin ED (2006) described the alternative method on lithium pretreatment, followed by one or several low doses of pilocarpine, produces status epilepticus (SE) and chronic epilepsy with much lower mortality rates than a single dose of pilocarpine. Pretreatment of lithium chloride (3mEq/ kg, i.p) between 2-24 hours prior to pilocarpine injection potentiates the epileptogenic action of pilocarpine and allows a 10-fold reduction in the drug dose.^[17]

Yohimbine model:

Antagonism against yohimbine-induced seizures in mice is considered to be a model predictive of potential GABA-mimetic agents. In mice the test compounds were administered intraperitoneally, 30 min prior to 45 mg/kg Subcutaneous route of yohimbine HCl. The animals are observed for the onset and number of clonic seizures for 60 min.^[14]

Bicuculline model:

Bicuculline has been applied focally and systemically. It has been used to induce acute simple focal epilepsy after topical application in the sensorimotor cortex in rats. Another model using bicuculline with induction of chronic simple partial seizures was developed by Remler and Coworker. This model mixes features of focal and generalized epilepsy and is referred to as systemic focal epileptogenesis. In this model, rats receive radiation to a limited volume (0.25 ml) of cerebrum. Three to six months later, when the blood-brain barrier is injected systemically, inducing an epileptic focus with recurrent EEG spikes and focal seizures enduring for several weeks after a single injection. The spikes are suppressed by phenytoin, Phenobarbital, chlorodiazepoxide and valproic acid.^[12]

Bicuculline is believed to exert its epileptogenic effect through blocking GABAergic neurotransmission by competing with GABA for its binding sites.^[12]

Kainic acid (KA) model:

Systemic administration of the appropriate dose of KA induces 'wet dog shakes', generalized tonic-clonic convulsions, teeth chattering and altered motor activity including an initial hypoactivity which transforms to a hyperactivity at later stage. Neurodegeneration occurs in the pyramidal layer of CA3 area of hippocampus and in the piriform cortex as early as 3 hours following injection. At this time point, a positive correlation exists between the dose of KA and the extent of the acute neurochemical changes including increases of 3, 4- dihydroxyphenylacetic acid and decrease in noradrenaline levels in all brain regions investigated. By 13 hours to 2 weeks, neuronal somata degenerate and disappear in areas such as the olfactory cortex and parts of the amygdaloid complex, hippocampal formation, thalamus and neocortex.^[18]

Penicillin model:

The convulsive properties of penicillin were first observed by Walker and Johnson. Chen et al established the effective dose of penicillin given i.v or i.p. in rats and cats to induce experimental seizures. In rats, i.p. injection of penicillin, 2.5-5.0 MU/kg, induces spikes after about 45 min and seizures after approximately 70 min. In cats, i.v administration of penicillin, 0.5-1.0 MU/kg induces spikes in on the average 10 min and seizures in 32 min, while i.p. administration of penicillin, 1-2 MU/kg causes spikes in 24 min and seizures in 72 min. It was suggested that the convulsive action of penicillin is based on its competition for GABA at the GABA receptor.^[12]

IN VITRO METHODS:

Intact hippocampal preparations or acute brain slices maintain much of the needed circuitry to generate electrographic seizures^[19, 20]. However, these preparations are typically only viable for several hours and thus are primarily useful only for studying acutely provoked seizures, not chronic epileptogenesis. Organotypic slice cultures, which can be maintained for weeks or longer, have been used to study a number of physiological and pathological processes in the brain, including circuit development, synaptic plasticity, and axonal sprouting^[21]. Slice cultures also afford the opportunity to investigate epilepsy. Most previous studies of organotypic slice cultures have focused on interictal spikes and electrographic seizures acutely provoked by convulsant drugs or other pharmacologic conditions over a relatively short time course.^[22-14] However, some studies have examined the development of spontaneous epileptiform activity over several weeks, which more closely mimic the process of epileptogenesis that occurs with in vivo animal models and human epilepsy.^[25, 26]

The recent study by Dyhrfeld-Johnsen et al. provides perhaps the most detailed characterization to date of the development and evolution of epileptiform activity within a chronic, in vitro model system. Extracellular field potential recordings were made from the CA3 region of organotypic hippocampal slice cultures between 7 and 30 days in vitro (DIV). A clear progression in epileptiform activity was observed over this time period, starting with predominantly interictal spike discharges around 14–17 DIV and transitioning to mostly ictal-like electrographic activity by DIV 25–30. Ictal events could last greater than 3 minutes in duration and were quite robust, occurring in at least 80% of slices.

While obviously lacking a behavioral correlate to the electrographic seizures, this in vitro model exhibits a number of characteristics that resemble in vivo epilepsy. After the initial preparation of the slice cultures, there is a latent period of at least a couple of weeks, during which no seizures are apparent and potential antiepileptogenic therapies could be targeted, similar to many types of acquired epilepsy. The developmental sequence of the subsequent onset of interictal spikes and progressive transition to ictal events closely mimics a similar temporal progression observed in the kainate model of epilepsy.^[27] Furthermore, the standard anticonvulsant drug, phenytoin, could suppress the ictal events, but not the interictal spikes, as commonly observed in human epilepsy.

One intended application of this study was to address the mechanistic relationship between interictal spikes and seizures. In particular, while interictal spikes could simply represent a marker of the epileptic brain, one interesting hypothesis is that interictal spikes cause the subsequent development of seizures, possibly by promoting synaptic synchronization and axonal sprouting^[28]. While the observation that the emergence of interictal spikes temporally precedes the onset of seizures is consistent with spikes causing seizures, this evidence only demonstrates correlation, not causation. More definitive testing of this

hypothesis would depend on the use of selective “anti-spike” agents, which currently do not exist.

The cellular and molecular mechanisms causing epileptogenesis in this in vitro slice culture model are not known and were not explored in this study. Presumably the initial act cutting the hippocampal slices represents a precipitating traumatic injury, which instigates the process of epileptogenesis, and thus this model can be viewed as an in vitro model of posttraumatic epilepsy. As a result of the severing of synaptic connections at the cut surfaces, massive axonal sprouting and synaptic reorganization represents a rational mechanism that could promote epileptogenesis in this model. This hypothesis could be readily tested with pharmacologic interventions that inhibit axonal sprouting, such as rapamycin. From a mechanistic standpoint, another remarkable feature of this model is that minimal additional interventions were necessary to trigger epileptogenesis. In most studies of acute slices or slice culture, a convulsant drug or other excitatory pharmacologic conditions are

needed to induce epileptiform activity. In this study, no such additional provocations were used; however, there was an interesting dependence of the epileptiform activity on the presence of the normal growth medium, as epileptiform activity disappeared quickly after switching from the growth medium to a standard artificial cerebrospinal fluid. The contribution of glutamine in the growth medium, with subsequent conversion to glutamate, was evaluated and did not appear to be critical in this study.

Future studies are required to determine the necessary co-factors that seem to promote epileptogenesis in this model. In any case, this study has unexpected implications for all research using similarly maintained organotypic slice cultures for other applications independent of epilepsy—the current report suggests that seizures could be an unrecognized confounding factor in other studies that aren’t necessarily focusing on epileptogenesis. Finally, this in vitro epilepsy model could have the largest impact in therapeutic applications.

Table 1: Common methods used to induce convulsion in animal models

Animal models	Methods to induce convulsion	Types of seizures
In vivo model	Electrical stimulation: Maximal electroshock(MES) Kindling Chemoconvulsants: Pentylenetetrazole (PTZ) Strychnine Picrotoxin Isoniazid Lithium pilocarpine Yohimbine Bicuculline 4-aminopyridine n-methyl d- aspartate Penicillin	Generalised tonic-clonic seizures Chronic partial seizures Myoclonic and absence seizures Acute simple partial seizures Acute simple partial seizures Clonic-tonic seizures Status epilepticus Clonic seizures Acute simple partial seizures Clonic-tonic seizures Status epilepticus Generalised tonic-clonic and absence seizures
In vitro model	Hippocampal slices GABA _A receptor binding Assay	Complex partial seizures
Genetic model	Photosensitive baboons Audiogenic seizures mice Totterer mice and seizures -prone mouse strains Genetically epilepsy- prone rats	Generalised tonic- clonic seizures

GENETIC ANIMAL MODEL FOR EPILEPSY:

The finding of seizure-prone animals led to the development of models that were thought to approximate human epilepsy more closely [29]. Several genetic models of epilepsy have been validated using clinically effective drugs, and these models are presently used in drug discovery programs.

Photosensitive baboons, Audiogenic seizure-prone mice (e.g., the DBA/2J), genetically seizure-prone rats (GEPR- and GEPR-9), Mongolian gerbils (*Meriones unguiculatus*), photosensitive fowl, tottering mice (tg/tg

strain), and epileptic dogs are the genetically used animal models. Several highly inbred strains of mice have been identified as a means to study the genetic basis of human epilepsy. Because the genetic constitution of the mouse is better known and can be manipulated more easily than that of other mammalian species, the mouse is an excellent animal model for genetic and biochemical studies of epilepsy [30].

Photosensitive baboons - *Papio papio*, from the casamance region of Senegal were first reported to suffer from photomyoclonic syndrome. Intermittent light stimulation at frequencies close to 25 flashes per seconds leads to seizures

characterized by eyelid, then face and body clonus and subsequently tonic spasms or full tonic-clonic convulsions^[31].

Audiogenic seizure-prone mice have proved useful for identifying potential AEDs. When these mice are exposed to high-frequency (10–20 kHz) and high intensity (90–120 dB) sound, they exhibit wild running, followed by generalized clonic-tonic seizures. With some seizure-susceptible strains of mice, the seizure focus must be established with a priming stimulus of high intensity sound. In several strains of mice, seizure susceptibility changes with age, usually reaching a maximal level between 2 and 4 weeks of life; however, the Frings and O'Grady strains of mice may retain their susceptibility to seizures into their adult life^[32].

The most commonly used strain of audiogenic seizure-prone mice for anticonvulsant identification is the DBA/2J. It is an inbred strain of the house mouse (*Mus musculus*), is the most studied strain of audiogenic seizure susceptible mice^[32].

The homozygous (tg/tg) strains totterer mice are prone to spontaneous epileptic seizures. These mice are recognized by a broad-based ataxia gait. By 3 to 4 weeks of age, they develop frequent partial and absence seizures. Spontaneous focal motor seizures occur a few times a day, manifested as unilateral clonic jerks of limbs with secondary generalization. Ninety-three percent of the seizures last for 15 minutes or longer. These seizures can be suppressed by diazepam.

Totterer mice also exhibit spontaneous petit mal seizures with synchronous 6-7 second spike-wave discharges in EEG. These spike-wave discharge last 0.3 to 10 seconds and occur hundreds of times per day and are accompanied by a behavioural petit mal seizure. These seizures are blocked by ethosuximide, diazepam and Phenobarbital while phenytoin is not effective^[31].

The quaking mouse, like the tottering mouse, has an autosomal recessive genetic disorder that produces tonic-clonic seizures. The homozygous mice exhibit tonic-clonic seizures when lifted by the tail and slowly rotated 180 degrees; mice handled in this manner exhibit seizures ~85% of the time. Drugs used to treat human absence seizures are less effective in this seizure model than are drugs used to treat focal motor seizures^[33].

E1 mice exhibit seizures in response to vestibular stimulation like tossing or spinning. Manifestations of seizures include limb and face automatism like chewing and salivation. There may be secondary generalization to tonic-clonic seizures. EEG recordings indicate onset of electrical discharges in deep limbic structures. Thus these mice can serve as model for complex partial epilepsy with secondary generalization. Phenytoin and phenobarbitone are effective in this model. Unfortunately, most genetic models of spontaneous seizures are presently impractical as a means of screening for new AEDs because of the limited availability of test animals and/or irregular seizure frequency

There are currently two separate and distinct colonies of genetically epilepsy-prone rats (GEPR), known as GEPR-3 and GEPR-9, which have been bred and used to identify AEDs. The two colonies differ primarily in the intensity of convulsions. The seizure pattern of the GEPR-3 consists of an initial running phase followed by clonic seizures. The GEPR-9 exhibit a seizure pattern similar to the DBA/2J mice, characterized by a terminal, complete tonic phase. Several neurochemical deficits occurring the GEPR that may have human correlates. Both noradrenergic and serotonergic deficits appear to be important in the seizure etiology of the GEPR^[34].

A specific inbred strain of Mongolian gerbil, *Meriones unguiculatus*, exhibits spontaneous motor seizures in response to a variety of stimuli. The motor and behavioral Characteristics, as well as the severity of the seizures, can be rated from grade 0 (no seizure) to grade 6 (tonic-clonic seizure progressing to death). The stimuli required to produce seizures are generally a novel environment and/or a stress-inducing incident. Several standard and newer AEDs have been shown to attenuate seizure occurrence in this model^[35]. Seizure susceptibility in domestic fowl is due to a homozygous autosomal recessive trait. The seizures are characterized by an upward and backward tonic extension of the head and neck, followed by a loss of leg muscle control. The final phase consists of violent wing flapping with clonic leg movements. The interictal EEG is characterized by high voltage slow-wave activity. Seizure incidence and severity are evaluated after administration of several of the clinically effective AEDs^[34].

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