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Emerging epilepsy models: insights from mice, flies, worms and fish

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Abstract

Purpose of review—Animal models provide a means to investigate fundamental mechanisms of abnormal electrical discharge (i.e., seizures). Understanding the pathogenesis of epilepsy and therapy development have greatly benefited from these models. Here we review recent mouse mutants featuring spontaneous seizures and simpler organisms.

Recent findings—New genetically engineered mice provide additional insights to cellular mechanisms underlying seizure generation (BK calcium-activated potassium channels and interneuron-expressed sodium channels), genetic interactions that exacerbate seizure phenotype (*Scn2a*, *Kcnq2* and background) and neurodevelopmental influences (*Dlx* transcription factors). Mutants for neuronal nicotinic acetylcholine receptors, Glut-1 deficiency and aquaporin channels highlight additional seizure phenotypes in mice. Additional models in *Caenorhabditis elegans* (Lis-1) and *Danio rerio* (pentylenetetrazole) highlight a reductionist approach. Taking further advantage of ‘simple’ organisms, antiepileptic drugs and genetic modifiers of seizure activity are being uncovered in *Drosophila*.

Summary—Studies of epilepsy in mutant mice provide a framework for understanding critical features of the brain that regulate excitability. These, and as yet undiscovered, mouse mutants will continue to serve as the foundation for basic epilepsy research. Interestingly, an even greater potential for analyzing epileptic phenotypes may lie in the more widespread use of genetically tractable organisms such as worms, flies and zebrafish.

Keywords

epilepsy; invertebrate; model; organism; seizure

Introduction

Epilepsy is a common neurological disorder characterized by recurrent self-sustaining bursts of abnormal electrical brain activity. Our understanding of how these bursts of electrical activity are generated, and more importantly how this activity can be reduced, has greatly benefited from animal model research. Traditional work in the epilepsy field relies on

chemical or stimulus-evoked paradigms to generate seizure activity and spontaneous epileptic phenotypes, largely in rodents. With the advent of genetically modified mice, reports of 'epileptic' mutant mice now total nearly 100 models in the current literature. In large part, these mutant mice feature a general inactivation (or 'knockout') of a specific gene and confirm much of what was already known about the delicate balance between excitation and inhibition in the central nervous system (CNS). New mice described in this review highlight seemingly lesser known pathways in the control of CNS excitability, genetic modifiers or signaling molecules that lead to subtype-specific cell loss and subsequent circuit modification. Where this review attempts to diverge from the many books and articles devoted to animal models [1,2*,3–5] is in the consideration of organisms not normally associated with epilepsy research. Although enjoying wide use in the general field of neurobiology, worms, fruit flies and zebrafish have only recently been explored as animal models of epilepsy. Some of these studies are described here. The goal of this brief review is not to dismiss traditional rodent models – indeed they remain important contributors to our overall understanding of seizures and epileptogenesis – but rather to put simpler organisms in the lexicon of epilepsy studies.

Gene interactions and novel epilepsy phenotypes revealed in genetically modified mice

In the past decade, the number of genetically modified mice exhibiting seizures (or reduced sensitivity to convulsant manipulations) has risen exponentially, with no clear endpoint in sight. The diversity of 'epilepsy' genes implicated in these mouse seizure phenotypes is staggering. From these mice ion channel, postsynaptic receptor, and genes required for neuronal migration were identified. Homologous recombination techniques remain the most popular method for gene inactivation in mice and two recently described ion channel knockouts present with interesting epileptic phenotypes. First, a $\beta 4$ subunit of the calcium-activated potassium (BK) channel was shown to control burst firing duration and spike frequency adaptation in dentate granule cells. Deletion of the $\beta 4$ subunit in mice resulted in nonconvulsive partial onset seizures [6**]. Second, inactivation of a voltage-gated sodium channel ($\text{Na}_v 1.1$) linked to severe myoclonic epilepsy in infancy (SMEI) reduced sodium current density in cultured hippocampal interneurons. $\text{Na}_v 1.1$ null mice exhibited ataxia and seizures early in the postnatal period progressing to periodic generalized clonic and bilateral forelimb convulsions by early adulthood [7]. Interestingly, video-electroencephalographic (EEG) recordings confirmed ictal activity patterns in roughly 40% of heterozygous mutants surviving into adulthood. Whether this incomplete phenotypic penetrance reflects compensatory changes in other sodium channel subunits ($\text{Na}_v 1.3$ was found to be upregulated) or background modifier genes (seizure phenotypes were more severe on a BL/6 background) or both, remains to be explored further.

In humans, Glut-1 deficiency is an autosomal-dominant disorder characterized by infantile seizures, developmental delay and ataxia. A recent study [8] indicated that homozygous deletion of GLUT-1 in mice is embryonic lethal (as expected) but heterozygous GLUT-1 mice exhibit interesting epileptic phenotypes. Not unlike other mice described in this section, video-EEG recordings were used to identify cortical activity patterns reminiscent of

generalized or partial lateralized seizures, bilateral spike-and-wave seizures or brief bilateral rhythmic spike discharges in GLUT-1 mutants. Novel seizure phenotypes were also described in a mouse featuring knock-in of a $\alpha 4$ neuronal nicotinic acetylcholine receptor associated with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) [9]. Thalamic cell cultures and synaptosomes prepared from heterozygous mice harboring an L9A' mutation were hypersensitive to nicotine. Although these mice were more sensitive to nicotine-induced seizure-like behavior no spike-wave discharges were observed in any of the brain regions examined.

Observations of variable epileptic phenotypes based on background strain differences were initially described by Schauwecker and Steward [10], and could explain some of the discrepancies described here. Further highlighting the notion of multiple gene interactions, a moderate epileptic phenotype observed in a transgenic mouse carrying a voltage-gated sodium channel mutation (*Scn2a*^{Q54}) associated with generalized epilepsy with febrile seizures plus (GEFS+) was shown to be enhanced when crossed into a *Kcnq2* mutant mouse background [11]. This same group [12*] also described a spike-and-wave absence seizure phenotype in backcrossed C3H/He mice, further demonstrating the digenic (and possible even polygenic) factors that may underlie the development of epilepsy. A long known role for inhibition in epilepsy was recently confirmed in a novel mutant mouse featuring inactivation of a transcription factor (*Dlx1*) necessary for interneuron development and migration [13]. Interestingly, cortical and hippocampal interneurons are initially present in normal numbers and locations in these mice but a subgroup of somatostatin, neuropeptide Y and calretinin immunoreactive interneurons die in early adulthood, leading to reduced GABA-mediated inhibition and generalized seizures. On the other hand, a less appreciated role for glial cells, redistribution of extracellular potassium ions and aquaporin water channels in seizure modification was demonstrated in a recent paper by Binder *et al.* [14]. Using aquaporin-4 null mice they found that stimulation-evoked seizure discharge was dramatically prolonged in these animals.

Seizure susceptibility and drug screening in fruit flies

Invertebrate model organisms such as *Drosophila melanogaster* (fruit flies) have played a central role in the discovery of ion channel mutations resulting in epileptic phenotypes. These channelopathy mutants include temperature-sensitive paralytic *seizure* (hERG), *slowpoke* (BK), and *Shaker* (Kv1) or ether-induced leg shaking *ether-a-go-go* (hERG) flies [15–17]. A larger class of bang-sensitive mutant progresses through distinct behavioral stages (muscle spasms and violent uncoordinated shaking, paralysis marked by lack of motion and responsiveness, delayed spasm and recovery) commonly interpreted as a seizure phenotype [18]. More importantly, physiological correlates of these behavioral stages can be monitored in recordings from the dorsal longitudinal flight motor neuron (DLM). In tethered flies, an electroconvulsive stimulus applied to the brain elicits an initial discharge of spikes, response failure, delayed discharge and recovery [18,19]. Interestingly, the electrical discharge pattern observed in flies resembles that described in kindling or afterdischarge stimulation protocols commonly used in rodents [20,21]. Given the similarities between *Drosophila* seizure phenotypes and epilepsy, it was only a matter of time before laboratories would begin using fruit flies in anticonvulsant drug screening strategies. In the first example,

the bang-sensitive mutant *easily shocked*² (ethanolamine kinase gene) was fed different concentrations of five conventional antiepileptic drugs. In a behavioral recovery paradigm, Reynolds and coworkers [22] identified phenytoin (dilatant) and gabapentin as effective anticonvulsant drugs. Carbamazepine, vigabatrin and ethosuximide were unable to ameliorate recovery in bang-sensitive mutants. In a second study, multiple compounds were tested in chemical convulsant *Drosophila* seizure models e.g., exposure to GABA_A antagonists picrotoxin or pentylenetetrazole [23*]. Phenytoin was again identified as a potential anticonvulsant in *Drosophila*, although this compound is not particularly useful in rodent picrotoxin/pentylenetetrazole models [24,25]. Using a *para* mutant, the authors also showed that the anticonvulsant activity of phenytoin requires a sodium channel α subunit. These studies suggest that *Drosophila* and the variety of readily available *Drosophila* P-insertion mutants [26], though logistically favoring high-throughput drug screening, may be best suited to identifying molecular sites of action for available antiepileptic drugs or seizure susceptibility genes (see below).

Although single gene mutations resulting in epileptic phenotypes were identified, these disorders are rare, and it is commonly accepted that multiple factors are at play in this disease [27]. Identification of gene interactions that exacerbate epileptic phenotypes is needed, and seminal work by Mark Tanouye and coworkers using *Drosophila* is already focused on this question. Generating double mutants in a bang-sensitive background, they recently identified several genetic modifiers of seizure activity [28,29]. While a behavioral paralysis protocol is described in these and related studies, the sensitivity and potential for quantification associated with their electrophysiological assay is more appealing to an epilepsy audience. The basic protocol is to measure the minimal voltage applied to the fly brain necessary to elicit a pattern of aberrant high-frequency neuronal firing followed by refractory (postictal) inactivity. This approach initially identified seizure susceptibility mutations in ethanolamine kinase, mitochondrial ribosomal protein, Shaker potassium channels and sodium channel genes [30]. Not unlike early mouse knockout studies and the widespread belief that epilepsy is an excitation–inhibition imbalance, identified genes play critical roles in the regulation of neuronal excitability. More recently, additional potassium and sodium channel mutants were described by Lee and Wu [19] and a potassium/chloride co-transporter mutant by Hekmat-Safe and coworkers [31*]. The latter finding, a fly homolog of KCC2, is of particular interest as co-transporter maintenance of intracellular chloride levels is critical to the depolarizing and hyperpolarizing functions of GABAergic interneurons [32]; antagonizing these co-transporters exerts antiepileptic effects *in vitro* and *in vivo* [33,34]. On the other hand, a caveat of this *Drosophila* approach is that phenotypes may be uncovered that are not necessarily epileptic. For example, the *couch potato* (*cpo*) mutant crossed into a bang-sensitive background exhibits an electrical phenotype similar to previously described seizure susceptible mutants, e.g. seizure stimulus thresholds less than half of wild type flies. These mutants also exhibit a synaptic transmission defect and the novel couch potato gene described, *cpo*^{EG1}, is linked to human neurodegenerative disorder spinocerebellar ataxia type I (SCA-1); ethanolamine kinase activity (e.g., bang-sensitive mutant used in seizure susceptibility studies) has also been linked to SCA-1 [35]. Whether ataxia or other neurological disorders accompanied by abnormal electrical discharge can be separated from epileptic phenotypes in *Drosophila* remains to be explored further.

Convulsions in a nematode model of lissencephaly

Lissencephaly is a rare childhood birth defect associated with schizophrenia, mental retardation and intractable epilepsy [36]. Although the ‘smooth brain’ pathology normally associated with lissencephaly-1 (*LIS1*) gene mutations cannot be reproduced in *Caenorhabditis elegans*, the genetic tractability of this organism offers a unique opportunity to study *LIS1* gene function. Nematodes (*C. elegans*) are transparent and have a well characterized nervous system consisting of precisely 302 neurons [37]. Homologs for a wide variety of proteins required for mammalian neurological function (e.g., ion channels, receptors, and neurotransmitters) are conserved in *C. elegans* [38]. Elegant electrophysiological techniques developed by Erik Jorgensen and colleagues to study individual neurons in nematodes [39] make this organism attractive to a field studying abnormal electrical discharge. Automated systems for recording and analyzing simple behaviors in *C. elegans* have also been established [40]. Given these possibilities, it was not surprising to see reports of *LIS1* gene function in *C. elegans*.

Initial studies of *C. elegans lis-1* gene expression focused on the localization to proliferating cells and neurons; developmental studies have shown that LIS1 proteins play critical roles in nuclear positioning, cell proliferation and chromosome segregation [41,42]. Two recent manuscripts from Guy Caldwell’s laboratory offer a more seizure-directed focus to these Lis-1 nematode studies. Using *pnm-1* (a nonsense allele of *lis-1*) homozygous animals that escape the early developmental lethality associated with LIS1 inactivation, digital video imaging experiments described ‘head-bobbing’ convulsions in *pnm-1* worms exposed to the common convulsant pentylenetetrazole. Although the simple neuronal architecture of *pnm-1* worms was not disrupted, irregularly sized and unevenly spaced puncta were observed [43]. These anatomical observations were interpreted as a defect in presynaptic vesicle transport within GABA-containing neurons and highlight an important advantage of this reductionist approach, namely, identification of LIS1 defects in synaptic structure that may be independent of neuronal migration deficits. To further demonstrate that LIS1-mediated nematode convulsions could be uncoupled from gross anatomical changes in brain structure, and were instead linked to intrinsic defects in synaptic vesicle transport, a second LIS1 worm manuscript was published in 2006 [44]. In this follow-up, RNA interference was used to inactivate several established LIS-1 pathway components and resulting mutants were then challenged with pentylenetetrazole. Worms depleted for NUD-1, NUD-2, DHC-1, CDK-5 and CDK-1 exhibited convulsive behaviors similar to pentylenetetrazole-exposed LIS1 mutants.

These studies provide an elegant example of how a simple organism can be exploited to uncover genetic modifiers of excitability (and ultimately epilepsy). Although pentylenetetrazole induced head-bobbing convulsions in *C. elegans* remain to be fully characterized as a seizure phenotype (specifically in terms of abnormal electrical discharge), these models may provide new insights to the fundamental mechanisms underlying convulsive behaviors.

Seizures in immature zebrafish

Although *Danio rerio* (zebrafish) are best known as a model organism to study development and vision, there is emerging interest in using these simple vertebrates in disease-based research [45,46]. Zebrafish offer a unique combination of genetic tractability, close homology with higher vertebrates and accessible experimental methodologies such as optical imaging, behavioral analysis, and electrophysiological recording. Given these possibilities, it was not a tremendous leap of logic when our laboratory decided to develop a zebrafish seizure model [47]. Using information accumulated from nearly a century of rodent seizure models, we exposed developing zebrafish larvae (6–7 days postfertilization) to various concentrations of pentylenetetrazole. Freely swimming zebrafish exposed to pentylenetetrazole in the bathing medium progressed from increased activity to rapid clonus-like full body convulsions followed by a brief loss of posture. Expression of an immediate early gene (*c-Fos*) upregulated in the CNS of many different rodent seizure models was also shown to be upregulated in the CNS of pentylenetetrazole-exposed zebrafish. More importantly, pentylenetetrazole exposure elicited brief small amplitude, high frequency ('interictal-like') and complex multispikes, large amplitude ('ictal-like') electrographic discharge that could be monitored using a field electrode placed in the zebrafish telencephalon or optic tectum. Pentylenetetrazole-evoked electrical discharges were similar in waveform to those reported in mice or rats injected with this drug [48], and were abolished by valproic acid and benzodiazepines in a concentration-dependent manner.

Although establishment and characterization of seizures in zebrafish is currently limited to an acute model, this organism offers several interesting possibilities for future studies. For example, zebrafish forward-genetic mutagenesis screens have uncovered many genes responsible for critical aspects of neurodevelopment and it is not difficult to imagine using this approach to identify new seizure susceptibility genes. Application of morpholino oligonucleotide gene knockdown (a rapid method to analyze gene function in developing larvae) [49] or transposon-mediated gene trapping (a simple means to create chromosomal insertions) [50] approaches offer more examples of how targeted gene inactivation schemes, perhaps guided by human gene mutations shown to cause epilepsy, could be exploited for the generation of novel zebrafish epilepsy models.

Conclusion

A considerable level of effort is devoted to debating and discussing animal models of epilepsy. As with many such debates, consensus on which models best reflect the human condition is difficult to achieve. Where emerging models may benefit the epilepsy field is in pushing our perspective toward novel genetic, cellular and anatomical hypotheses. If one follows the latter philosophy, then a more appropriate question in evaluating emerging animal models is 'will this model answer a specific epilepsy question?' and not 'how closely does the model mimic the human condition?'

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Abbreviation

CNS central nervous system

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