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From bench to drug: Human seizure modeling using *Drosophila*

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Abstract

Studies of human seizure disorders have revealed that susceptibility to seizures is greatly influenced by genetic factors. In addition to causing epilepsy, genetic factors can suppress seizures and epileptogenesis. Examination of seizure-suppressor genes is challenging in humans. However, such genes are readily identified and analyzed in a *Drosophila* animal model of epilepsy. In this article, the epilepsy phenotype of *Drosophila* seizure-sensitive mutants is reviewed. A novel class of genes called seizure-suppressors is described. Mutations defining suppressors revert the “epilepsy” phenotype of neurological mutants. We conclude this review with particular discussion of a seizure-suppressor gene encoding DNA topoisomerase I (*top1*). Mutations of *top1* are especially effective at reverting the seizure-sensitive phenotype of *Drosophila* epilepsy mutants. In addition, an unexpected class of anti-epileptic drugs has been identified. These are DNA topoisomerase I inhibitors such as camptothecin and its derivatives; several candidates are comparable or perhaps better than traditional anti-epileptic drugs such as valproate at reducing seizures in *Drosophila* drug-feeding experiments.

1. Introduction

Drosophila has been a model for examining fundamentally important problems in biology, especially developmental biology and neurobiology (Rubin and Lewis, 2000). A lesson from these studies is that findings are generally applicable to other experimental model systems such as *Caenorhabditis elegans* nematodes and mice due to conservation of fundamental processes and essential gene products (Veraksa et al., 2000; Tickoo and Russell, 2002). An implication from cross-species conservation is that *Drosophila* has the potential to be a powerful system for modeling human pathologies. This comes, in part, from estimates of 75% of all human disease genes have related sequences in *Drosophila* (Bier, 2005). *Drosophila* models have been developed for cancer, cardiac disease, and several neurodegenerative diseases such as Parkinson's disease, Huntington's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (reviewed in Bier and Bodmer, 2004; Bier, 2005; Michno et al., 2005; Vidal and Cagan, 2006). Here we review *Drosophila* modeling of human seizure disorders.

Human seizure disorders are a significant health concern due to the large number of affected individuals, the potentially devastating ramifications of untreated seizure episodes, and the limitations of antiepileptic drug (AED) options. Seizure-suppressor genes provide a powerful tool for examining seizure disorders and identifying potential AED targets. The major interest

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in seizure-suppressors is that they may lead to new and significant treatments for human epilepsy. Seizure-suppressor genes could help define targets for unexpected classes of anticonvulsant drugs that are effective new treatments for epilepsy: treatments for intractable syndromes or treatments with reduced side effects. Another possibility is to discover candidate genes that might be used for gene therapy. Among the several questions that arise are: what are seizure-suppressor genes and how might they lead to new therapeutics? What is the entire range of potential gene products that can act as seizure-suppressors? Is this range limited to nervous system-specific gene products, such as signaling molecules or does it include non-nervous system gene products as well? This article focuses on a *Drosophila* model of epilepsy, illustrating the use of genetic screens to identify seizure-suppressor genes and their potential applications to therapeutics.

2. The utility of *Drosophila* in studying human seizure disorders

2.1. Animal models of epilepsy

Numerous animal models have been utilized to study epilepsy. Some interesting but uncommon models include baboon, chicken, cat, dog, and Mongolian gerbil (Avoli, 1995; Bertorelli et al., 1995; Menini and Silva-Barrat, 1998; Batini et al., 2004; Lohi et al., 2005). More recently, the model genetic organisms zebrafish and *C. elegans* have been shown to be valuable in study of seizure disorders (Baraban, 2007). Zebrafish larvae exhibit mammalian-like seizure activity when administered the convulsant drug, pentylenetetrazole (PTZ) (Baraban et al., 2005). PTZ-treated larvae dart around the culture dish, swim in circles, convulse, and then paralyze for several seconds. This behavior is coupled with abnormal brain electrophysiology as recorded using fish electroencephalography, revealing ictal and interictal bursts of neuronal firing during seizure activity. The behavior has been successful in genetic screening for seizure-resistant mutant fish, identifying six such resistant mutants (Baraban, et al., 2007). *C. elegans* is used to model epilepsy caused by lissencephaly. Worms with a mutated *LIS1* gene are more susceptible to PTZ-induced convulsions than normal (Williams et al., 2004). Furthermore, worms depleted for *LIS1* pathway components in the worm show genetic interactions that greatly enhance sensitivity to convulsions (Locke, et al., 2006).

Mouse models of epilepsy have been shown to recapitulate many aspects of seizure disorders in humans (Noebels, 2003). Epileptic mice exhibit a variety of spontaneous seizure phenotypes including generalized tonic-clonic seizures and non-convulsive absence seizures. Seizures have an electrophysiological correlate in electrographic recordings from the brains of epileptic mice. In addition to phenotypic similarities, there are genetic similarities between human and mouse epilepsies. Numerous human epilepsy genes cause epileptic phenotypes in mice. Similar to humans, epilepsy genetics in mice frequently follow non-Mendelian, complex inheritance patterns, such as in the case of the EL model of mouse epilepsy (Legare et al., 2000).

In spite of its excellence as a phenotypic and genetic model of human epilepsy, the mouse has some experimental limitations. It is difficult to generate and screen for new mutants. Likewise, it is expensive and labor-intensive to maintain and manipulate large numbers of animals. An alternative model is the fruitfly *Drosophila melanogaster*. Several features make *Drosophila* an attractive experimental system. Chemical and transposon-based mutagenesis methods, drug-feeding capabilities and behavioral analyses have large capacity for examining large numbers of experimental animals. Advanced electrophysiology methods and molecular genetic techniques greatly facilitate mutant analysis. Epilepsy mutations, seizure suppressors, and seizure enhancers may be studied in single, double, and triple mutant combinations to gain insights into Mendelian and non-Mendelian inheritance patterns. Many of these capabilities are also available in other genetic model organisms such as *C. elegans* and zebrafish. Here we focus or recent progress made using the *Drosophila* model.

2.2. Seizure-like activity in adult Drosophila

Electrical shock of sufficient intensity delivered to the brain of *Drosophila* elicits seizure-like activity (Engel and Wu, 1994; Pavlidis and Tanouye, 1995; Lee and Wu, 2002), similar to all animals with complex nervous systems, including humans. Stimulus is a short wavetrain of high frequency electrical impulses (HF stimulation). Seizure-susceptibility is quantified by seizure threshold: the voltage that HF stimulation becomes an electroconvulsive shock (ECS) and elicits seizure-like activity (Kuebler and Tanouye, 2000). In *Drosophila*, seizure-like activity can be elicited by HF brain stimulation in all genotypes tested, including seizure-sensitive mutants, wild type, and seizure-suppressor mutants (Kuebler and Tanouye, 2000; Kuebler, et al., 2001). However, seizure-sensitive mutants exhibit seizure thresholds that are much lower than wild-type controls (Kuebler and Tanouye, 2000; Kuebler et al., 2001). *Drosophila* seizure-like activity is manifest as uncontrolled, abnormal HF neuronal firing that approaches 100 Hz for 3 s. Seizure-like activity appears to be extensive with all neurons thus far examined participating in the seizure. This is about 30 different excitatory motoneurons that apparently transmit glutamate (Kuebler and Tanouye, 2000). Immediately following seizure-like activity is a period of synaptic failure where transmission is interrupted at many central chemical synapses (Fig. 1). Experimentally, motoneurons innervating dorsal longitudinal muscles (DLMs) are monitored to track the occurrence of seizure-like activity and the time course of synaptic failure.

2.3. Drosophila seizure-sensitive mutants

A collection of eleven seizure-sensitive mutants called bang-sensitive (BS) paralytic mutants is the basis for a *Drosophila* seizure model (Table 1). Seizure-like behaviors are prominent in BS mutants. Following a mechanical shock, such as a tap of the culture vial on the bench top or brief vortex mixing (a ‘bang’), mutants undergo seizure-like behaviors characterized by initial seizure (2 s), temporary paralysis (20-300 s), and recovery seizure (2 s) (Benzer, 1971;Ganetzky and Wu, 1982)(Fig. 1). In addition to mechanical bang stimulation effectiveness, seizure-like behavior in mutants is also elicited by visual stimulation, in particular, stroboscopic light stimulation (R. Hoy, personal communication).

The canonical BS mutants include *bang senseless* (*bss*), *bang sensitive* (*bas*), *easily shocked* (*eas*), *slamdance* (*sda*), *technical knockout* (*tko*), and *knockdown* (*kdn*). For most of these canonical mutants, the BS behavioral phenotype is completely penetrant and electrophysiological seizure threshold is low, usually below 7 V (Kuebler and Tanouye, 2000). In comparison, normal flies never display a BS behavioral phenotype and have electrophysiological seizure thresholds usually greater than about 35 V (Kuebler and Tanouye, 2000). The molecular basis of *bss* and *bas* phenotypes remains unknown. The genes for *eas*, *sda*, *kdn* and *tko* have been cloned and characterized (table 1). We have settled on three mutants as experimental representatives of the collection. 1) The *bss* mutant, a severe BS mutant, is behaviorally and electrophysiologically the most sensitive to seizure with a prominent tonic-clonic phenotype. It is also the most difficult BS mutant to suppress genetically and pharmacologically. 2) The *eas* mutant displays moderate seizure-sensitivity; the gene encodes ethanolamine kinase. 3) The *sda* mutant displays weak seizure-sensitivity in that it possesses a phenotype that is fully penetrant, but is the easiest to suppress genetically and pharmacologically; the *sda* gene encodes aminopeptidase.

As example, the *eas* BS mutation is described in more detail. Flies carrying *eas* have a defect in the ethanolamine kinase gene, which interferes with the metabolism of phosphatidylethanolamine, the predominant membrane lipid in *Drosophila* (Pavlidis et al., 1994). The *eas* allele that causes the BS behavioral phenotype is eas^{PC80} containing a 2-bp deletion that introduces a frame shift; the resulting truncated protein lacks a kinase domain and is devoid of enzymatic activity (Pavlidis et al., 1994). The BS behavioral phenotype of *eas*

flies is completely penetrant, 100% of flies are paralyzed by mechanical stimulation. The seizure threshold of *eas* is very low compared to the wild-type (3.4 V, about 10-fold lower than wild-type), making *eas* a desirable genetic background used to screen for seizure-suppressors. The *eas* defect may cause increased seizure-susceptibility by affecting the function of integral membrane proteins such as ion channels or by affecting membrane fusion events such as neurotransmitter secretion. A recent study showed that eas^{PC80} also has a mushroom body defect similar to the eas^{ala} allele, pointing to a new role for *eas* in developmental mushroom body neuroblast proliferation (Pascual et al., 2005).

Several non-canonical BS mutants have been identified including *couch potato* (*cpo*) and *kazachoc* (*kcc*). These mutants are "non-canonical" in the sense that, unlike most of the original BS mutants, their BS phenotype is incompletely penetrant and their seizure thresholds tend to be higher (11-16 V), but still below wild-type levels. Despite this weaker BS phenotype, the non-canonical BS mutants appear to model epilepsy well, perhaps better than the canonical BS mutants. They are especially strong enhancers of seizure with prominent effects in genetic interaction. The *cpo* mutant is a P-element mutation that was isolated in a screen for new BS mutants that utilized *sda*/+ heterozygotes as a sensitized genetic background (Glasscock and Tanouye, 2005; Zhang et al., 2002). The *cpo* mutation disrupts an RNA-binding protein leading to a variety of neurological phenotypes including behavioral abnormalities, seizure-susceptibility, and synaptic transmission defects (Glasscock and Tanouye, 2005). The *cpo* phenotype is reminiscent of the majority of human seizure disorders, which exhibit symptomatic epilepsy as one of a set of complex neurological symptoms. The *kcc* mutation was identified as a spontaneous mutation present on the second chromosome of a *sda*-enhancer stock. The mutation renders flies susceptible to epileptic-like seizures due to reduced expression of the K^+/Cl^- cotransporter gene (Hekmat-Scafe et al., 2006). *Drosophila kcc* mutations resemble those described for mouse KCC2. Seizure phenotypes are observed in mouse KCC2 knock-down mutants that reduce the normal level of neuronal K^+/Cl^- cotransporter (Woo et al., 2002; Tornberg et al., 2005). In the more severe knock-down mutant (5% normal KCC2 level), generalized seizures are frequently induced by the mild mechanical stimulation that occurs during handling (Woo et al., 2002).

2.4. Comparisons of seizure-like activity in flies and human seizures

Seizures in flies and humans have several similarities providing support for the utility of this type of investigation. Previous investigations have shown that for *Drosophila*: (1) all individuals have a seizure threshold; (2) genetic mutations can modulate seizure susceptibility; (3) electroconvulsive shock treatment (ECT) in flies raises the threshold for subsequent seizure-like activity; (4) seizure-like activity spreads through the fly central nervous system (CNS) along particular pathways that are dependent on functional synaptic connections and recent electrical activity; (5) seizure-like activity in flies can be spatially segregated into particular regions of the CNS; (6) *Drosophila* phenotypes can be ameliorated by the human AEDs sodium valproate, phenytoin, gabapentin, and potassium bromide; and (7) mutations affecting *Drosophila* sodium channels are excellent seizure suppressors, consistent with the notion that many AEDs are targeting sodium channels (Kuebler and Tanouye, 2000, 2002; Kuebler et al., 2001; Reynolds et al., 2003; Tan et al., 2004).

An especially interesting phenomenon during fly seizure episodes in BS mutants is that upon recovery, the fly is resistant to subsequent BS paralysis for several minutes to over an hour depending on genotype, a time termed the refractory period (Ganetzky and Wu, 1982). This surprising effect is of particular interest because, although mutations causing paralysis were known in the literature, a refractory period for a behavioral defect was unheard of and novel. It has since become clear that all BS mutants have a refractory period (Ganetzky and Wu, 1982), and this has become one of their defining features. Electrophysiologically, flies possess

seizure thresholds that are transiently elevated during the reractory period (Pavlidis and Tanouye, 1995; Kuebler and Tanouye, 2000). In humans, refractory period is observed following electroconvulsive shock treatment (ECT) that is used for treatment of epilepsy and depression in the clinic. ECT has numerous anticonvulsant effects, including elevated seizure threshold and decreased seizure duration, which makes it a useful adjunctive therapy in epilepsies that are not amenable to treatment with medication.

3. Genetic suppression of seizure-susceptibility in *Drosophila*

3.1. Identification of seizure-suppressor mutants

Human seizure disorders represent a pervasive class of disease with unsatisfactory treatment options. In addition, a large number of disparate genes are involved in epileptogenesis. Frequently, there is a lack of obvious functional relationship between mutation and seizure susceptibility, complicating an understanding of epilepsy on a mechanistic level. Seizure-suppressor genes provide a potentially powerful tool for examining seizure disorders and identifying potential AED targets. The general approach to identify suppressors is straightforward: starting with an epilepsy genetic background, second-site suppressor mutations are evaluated for their ability to revert the epilepsy phenotype to wild type. Surprisingly, the basic approach of utilizing second-site suppressor mutations has not been extensively exploited for neurological syndromes.

The most promising aspect of the approach is the apparent abundance of seizure-suppressor mutations and ease of identifying them. Reverse genetics utilizing double mutant combinations, as well as forward genetics screens utilizing a variety of mutagenesis schemes available for *Drosophila* have all been used productively to isolate seizure-suppressor mutations. Reverse genetics searches focusing on existing *Drosophila* nervous system excitability mutations have shown that sodium channel (*para*, mle^{napts}), potassium channel (*Sh*), and gap junction mutations (*shakB*) are all effective seizure-suppressors (Kuebler et al., 2001; Song and Tanouye, 2006). Seizure-suppressor mutations have also been readily isolated in forward genetics mutant screens. Especially interesting are novel and unexpected seizure-suppressor mutations, not obviously affecting electrical signaling. These include DNA topoisomerase I enzyme ($top1^{JS1}$), a zinc-finger transcription factor *escargot* (*esg*), and the meiotic gene $mei\text{-}P26^{EG}$ (Song et al., 2007; Hekmat-Scafe et al., 2005; Glasscock et al., 2005). The following is a more detailed description of selected seizure-suppressor mutations. Continued identification of seizure-suppressor mutations through genetic screens will provide a rich source of genetic aberrations that can serve to dissect apart seizure-susceptibility and act as a basis for discovering new AED targets.

3.2 Insights into seizure-suppression mechanisms from mutant analysis

3.2.1 Seizure-suppression by sodium channel mutations—Mutations affecting voltage-gated sodium channels are potent seizure-suppressors and provide validation for the suppressor genetic approach. Reverse genetic experiments were inspired by observations that sodium currents are major targets for several first-line AEDs including phenytoin, carbamezapine, lamotrignine, and valproate (Ragsdale and Avoli, 1998; Catterall, 1999). Two Drosophila sodium channel mutants are $para^{ST76}$ and mle^{napts} that affect the channel structural gene and regulate channel expression, respectively (Ramaswami and Tanouye, 1989; Loughney et al., 1989; Kuroda et al., 1991). Both mutants are seizure-resistant mutants showing ECS seizure thresholds that are about 2-3 times higher than for wild type animals (Kuebler et al., 2001). Double mutant combinations between each of the sodium channel mutants and various BS mutants show a reversion of seizure-susceptibility to wild type levels (Kuebler et al., 2001).

The *para*^{ST76} and *mle*^{napts} mutations exert their effects through a reduction of functional sodium channels (Loughney et al., 1989; Kuroda et al., 1991). This suggests that they inhibit seizures in much the same way as the AEDs phenytoin and carbamazepine (Kuo, 1998; McNamara, 1999). During repetitive firing, these drugs are thought to stabilize sodium channels in the inactive state thereby reducing the number of channels that can be activated. This leads to reduced capacity for HF firing (McLean and MacDonald, 1983; 1986). Indeed, reduced capacity for HF firing has been observed in *para*^{ST76} and *mle*^{napts} mutants (Nelson and Wyman, 1990; Kuebler et al., 2001).

In a forward genetic screen by mutagenesis using a DNA transposable element, a new *para* allele *para*^{JS} was identified with a transposon insertion in the 3′-UTR (3′ untranslated region) of the *para* gene (Song and Tanouye, 2007). The *para*^{JS1} mutation is not only exceptionally effective as a seizure suppressor, comparable to *para*^{ST76} and *mle*^{napts}, but also shows no obvious side-effect phenotypes whether in a wild-type or an *eas* mutant genetic background. That is, *para*^{JS1} mutant flies have no evidence of sluggish behavior; they show excellent fertility; and they have normal-length life span, unlike other previously identified *Drosophila* sodium channel mutants. Other behaviors also appear to be normal: grooming, courting, mating, jumping and flying. Identification of the new *para* allele validates the forward genetic approach, providing evidence that it recapitulates suppressors identified by reverse genetics.

3.2.2. Gap junction mutations suppress seizure-like activity—Electrical synaptic transmission through gap junctions is an important mechanism for synchronizing signaling in the brain, combining together with field effects, chemical synaptic transmission mechanisms, and ionic channel mechanisms in the generation and maintenance of seizures (Carlen et al., 2000). Experimental observations reinforce the notion of gap junction contributions to seizures. Pharmacology that reduces electrical transmission diminishes seizures, and enhanced electrical transmission increases the frequency and severity of seizures (Carlen et al., 2000; Jahromi et al., 2002). For example, carbenoxolone, a gap junction blocker, has been shown to reduce seizures in several animal models of epilepsy (Gajda et al., 2003; Jahromi et al., 2002).

Gap junction proteins in *Drosophila* are encoded by a family of eight genes called the “innexin” family (Phelan and Starich, 2001). The *shakB*^{2} gene encodes a gap junction protein and mutations in this gene have impaired electrical transmission (Krishnan et al., 1993; Phelan et al., 1996). One allele, *shakB*^{2}, is a loss-of-function mutation in which a T to A substitution inserts a stop codon within the signal sequence (Zhang et al., 1999b). The *shakB*^{2} mutant is a seizure-resistant mutant showing an ECS seizure threshold that is about three times higher than for wild type animals (Kuebler et al., 2001). Double mutant combinations between *shakB*^{2} and various BS mutants show that it is an effective seizure-suppressor mutation: it completely suppresses seizure-like activity caused by *sda*, *kdn*, and *jbug* mutations. Seizure-like activity caused by *eas* and *tko* mutations are partially suppressed by *shakB*^{2}. Seizure-like activity caused by *bas*^{2}, *bss*^{1} and *bss*^{2} mutations are not suppressed by *shakB*^{2} (Kuebler et al., 2001; Song and Tanouye, 2006). Based on these interactions with *shakB*^{2}, it is determined that *sda*, *eas* and *bss* are representative of the entire BS collection. Synapses in the *shakB*^{2} mutant have been shown to be impaired in electrical transmission (Thomas and Wyman, 1984; Phelan et al., 1996). Thus, *shakB*^{2} is proposed to suppress seizure-like activity by a mechanism similar to that suggested for drugs such as carbenoxolone that block gap junction activity (Szente et al., 2002). Impaired transmission at electrical synapses is thought to interfere with synchronous activation of neuronal populations leading to decreased seizure susceptibility. Observations on *shakB*^{2} are generally consistent with this mechanism.

3.2.3 Seizure-suppression by mutation of DNA topoisomerase I—A major interest in seizure-suppressor mutations comes from the potential to develop new epilepsy therapeutics.

Novel targets can be discovered for developing drugs that can complement presently available first-line AEDs: new treatments for intractable epilepsy or drugs with reduced side-effects. Also of great interest are genes that may be candidates for use in gene therapy. Gene therapy approaches are not well-developed for treatment of epilepsy and this possibility could initiate from study of seizure-suppressor genes. Genetic screens have identified several unexpected mutations that are surprisingly effective seizure-suppressors. These mutations are unexpected because in each case the gene product is not obviously involved in any type of neuronal signaling. The expectation is that drugs developed from these may lack the deleterious nervous system side-effects commonly associated with AEDs.

New seizure-suppressor mutations include the *mei-P26*^{EG} mutation. The *mei-P26*^{EG} suppressor affects an interesting protein known as a ring finger B-box coiled-coil-NHL protein (Glasscock et al., 2005). The suppressor phenotype appears to result from missense mutation of a critical amino acid residue in the NHL protein-protein interaction domain of the protein. The suggestion from this result is that there may be proteins that are interaction partners of *mei-P26*^{EG} that are additional seizure-suppressors waiting to be identified. The zinc-finger transcription factor *esg* is another novel seizure-suppressor mutation identified by mutant screening. This is a gain-of-function mutation that suppresses seizure-like activity when the normal product *(esg*$^{+}$*)* is over-expressed ectopically in all nerve cells (Hekmat-Scafe et al., 2005). Gain-of-function mutations such as *esg* have qualities that make them candidates for gene therapy development.

Especially interesting for AED development is discovery of a new allele of *Drosophila* DNA topoisomerase I named *top1*^{JS1} (called topo I in mammals) (Song and Tanouye, 2007). The *top1*^{JS} allele was isolated in a seizure-suppressor screen that used the DNA transposable element, P-element, as a mutagen. Suppression was caused by insertion of the P-element in the 5′ untranslated region of the *top1* gene: 257 bp upstream of the translation start site. Seizure suppression is due to reduced transcription of the *top1* gene: mutant transcription is about 12.5-fold less than normal. The *top1* gene is an essential gene: of several known *top1* alleles, *top1*^{JS} is the only viable mutation, while all others are homozygous lethal (Lee et al., 1993; Zhang et al., 2000). The *top1*^{JS} mutation is a general seizure-suppressor ameliorating phenotypes of *sda, eas*, and *bss* (Song and Tanouye, 2007). As example, *top1*^{JS} suppresses *sda* seizure-like behaviors and paralysis by about 73%. The threshold for evoking seizures by ECS is raised about 2.5 fold. For *eas*, behavioral phenotypes are suppressed in 63% of animals and seizure threshold is raised about 3.5 fold. The *bss* seizure and paralytic behavior are not suppressed by *top1*^{JS} significantly. That is, most *top1*^{JS} *bss* double mutants showed bang-sensitivity. However, there is some indication that *top1*^{JS} acts to reduce the severity of *bss* seizure-like behavior, mainly about a two-fold decrease in tonic-clonic-like activity (Song and Tanouye, 2007). Taken together, these results are consistent with *top1*^{JS} being a general seizure-suppressor: with *sda* and *eas* mutants more easily suppressed; and *bss* mutants more resistant to suppression.

4. Anticonvulsant drug development from seizure-suppressor genes

4.1. Anticonvulsant drug testing in Drosophila

One type of validation of a model for epilepsy therapeutics comes from an assurance that currently available human treatments are effective on *Drosophila* seizure mutants. Previous discussions in this review compared *Drosophila* and human seizure disorders behaviorally and electrophysiologically. Especially pertinent are studies showing sodium channel mutants are excellent seizure suppressors because voltage-gated sodium channels are targets of several AEDs (Kuebler et al., 2001; Song and Tanouye, 2007). Further validation for the use of *Drosophila* in drug development comes from experiments using valproate (Kuebler and Tanouye, 2002). Valproate injected directly into the *Drosophila* brain is a very potent AED:

ECS seizure threshold in *bss* and *sda* mutants is immediately elevated to levels comparable to wild-type controls.

There is considerable interest in using *Drosophila* for high-throughput AED screening (Reynolds et al., 2003; Tan et al., 2004; Stilwell et al., 2006). The most convenient method is to feed seizure-sensitive *Drosophila* mutants a panel of drugs to be screened and then select for reversion of seizure-like behaviors or paralytic behavior. Promising drugs could then be examined subsequently in more detail by electrophysiological methods, perhaps by direct injection in the brain as for valproate (Kuebler and Tanouye, 2002). AED efficacy in feeding experiments has been by measuring reduction in paralytic recovery time for *bss* or *eas* mutants (Reynolds et al., 2004; Tan et al., 2004; Song and Tanouye, 2006). The AEDs phenytoin, gabapentin, potassium bromide, and carbenoxolone have all been deemed effective in *Drosophila* based on reduction of BS mutant recovery time. Using these criteria, carbamazepine, ethosuximide, and vigabactrin do not appear to be effective AEDs when fed to BS mutant *Drosophila* (Reynolds et al., 2004). AED drug-feeding experiments have generally not seen dramatic changes in percentages of BS paralysis or dramatic improvements in seizure threshold, although phenytoin may be an exception (Reynolds et al., 2004; Tan et al., 2004; Song and Tanouye, 2006).

4.2. AED development from the top1 seizure-suppressor

4.2.1 An AED target inspired by top1 seizure-suppressor—Identification of the $top1^{JS}$ mutation as a seizure-suppressor has led to consideration of a class of DNA topoisomerase I inhibitors as potentially effective AEDs. These drugs, called top1 (or topo I) inhibitors, have been found to be effective at ameliorating some seizure phenotypes in the *Drosophila* model (figure 3). DNA topoisomerase I is an essential nuclear enzyme involved in relieving the torsional stress associated with DNA replication, transcription, and chromatin condensation (Champoux, 2001). In this respect, it differs substantially from the targets of presently available AEDs, mainly sodium channels and GABA-related proteins. The $top1^{JS}$ seizure-suppression is consistent with the observation from molecular studies that many syndromes presenting with epilepsy, including human syndromes, mouse knockout mutations, and *Drosophila* mutations, are not obviously affecting electrical excitability functions (Royden et al., 1987;Pavlidis et al., 1994; Purnamm and McNamara, 1999; McNamara, 1999,Zhang et al., 2002). One might similarly expect that many seizure-suppression mechanisms would exist that are not working via alteration of electrical excitability. It may be possible to gain new insights into mechanisms by which the nervous system can be constructed in ways that reduce seizure sensitivity independent of effects on electrical excitability.

4.2.2 Top1 inhibitors as AEDs suppress Drosophila phenotypes—Top1 inhibitors are the first neurological drug candidates arising from a suppressor genetic approach. Top1 inhibitors have recently drawn considerable pharmacological interest, albeit not as neurological drugs, but as chemotherapeutic drugs in the cancer clinic (Wang et al., 1997; Pommier et al., 1999; Li and Liu, 2000). The top1 inhibitor camptothecin (CPT) is a potent anticancer agent, and its derivatives topotecan and irinotecan have been approved by FDA for treatment of ovarian and colorectal cancer (Mathijssen et al., 2002). Type 1 DNA topoisomerase enzyme is thought to resolve DNA torsional tension by binding DNA and relaxing the helix. After DNA relaxation, top1 enzyme is cleaved and the DNA is religated. CPT top1 inhibitor is thought to work by covalently binding to the top1-DNA complex forming a ternary complex that interferes with religation. This CPT religation interference can lead to apoptosis (Leppard and Champoux, 2005). CPT-induced apoptosis may be essential to its action in cancer therapy and may also contribute to its function as an AED (Song et al., 2007).

CPT, apigenin, and kaempferol are plant phytochemicals that inhibit top1 activity (Pommier et al., 1998; 1999; Snyder and Gillies, 2002; Boege et al., 1996). These top1 inhibitors have been shown to ameliorate phenotypes in BS mutants indicating that they have actions similar to AEDs in the *Drosophila* epilepsy model. For example, bang-resistant behavior is observed in a small number of *eas* mutants (3.5% of animals) that are reared in culture medium containing apigenin (drug-rearing experiments) (Song et al., 2007). Although a fairly modest reversion, nonetheless this is better than for most other AEDs thus far examined by feeding experiments, including valproate, and potassium bromide (Kuebler and Tanouye, 2002; Tan et al., 2004), although may be less effective than phenytoin (Reynolds et al., 2004). Top1 inhibitors reduce the paralytic recovery time of BS mutants in drug-rearing experiments and in short-term drug feeding experiments, resembling the effects of AEDs in the *Drosophila* model (Song et al., 2007; Kuebler and Tanouye, 2002; Tan et al., 2004; Reynolds et al., 2004). For example, *bss* flies fed CPT in short-term drug feeding recover from paralysis about two-thirds faster than control flies (Song et al., 2007). In addition, tonic-clonic-like activity is nearly completely suppressed. Electrophysiological recordings corroborate the behavioral results. Drug treatment causes a modest albeit significant increase in seizure threshold. Synaptic failure time is greatly decreased (Song et al., 2007). A comparison of drug feeding experiments in the *Drosophila* suggests that, in general, top1 inhibitors may be a less effective anti-epileptic agent compared to phenytoin, but may be better than valproate, potassium bromide, and carbenoxolone (Kuebler and Tanouye, 2002; Reynolds et al., 2004; Tan et al., 2004; Song and Tanouye, 2006). In addition, top1 inhibition appears to be tolerated much better than valproate with considerably less toxicity. Further experiments in mammalian animal model systems will provide insights into the pharmacological perspectives to evaluate top1 as an anticonvulsant compared to currently available AEDs on the market.

5. Conclusions

A major obstacle in neurological drug discovery comes about because of the limitation of organism-based drug screening that is presently a fairly low throughput assay. For example, screening and testing of drugs for epilepsy can be primarily dependent on laborious tests of rodents utilizing convulsants such as pentylenetetrazol or picrotoxin to induce seizure (White et al., 2002; Yang and Frankel, 2004). Here, we report on an alternative approach that relies on *Drosophila* seizure-suppressor genes to identify potential AED targets. This approach has, thus far, proven to be surprisingly productive mainly due to the ease with which novel seizure-suppressor mutations have been identified. Using this approach, a new, novel class of potentially effective AEDs may have been discovered, top1 inhibitors including CPT and related drugs. This is a promising first try using the power of the *Drosophila* seizure-suppressor gene approach. The hope is that continued identification of seizure-suppressor genes will lead to even more promising AED candidates. If further tests confirm the use of top1 inhibitors and other promising AED candidates in the neurology clinic, we suggest *Drosophila* will have a major impact on directly improving the human condition through advances of medical treatments in nervous system and other debilitating disorders.

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Abbreviations

AED
: antiepileptic drug

BS
: bang-sensitive *Drosophila* mutant

CNS
: central nervous system

CPT
: camptothecin

DLM
: dorsal longitudinal muscle

ECS
: electroconvulsive shock

ECT
: electroconvulsive shock treatment

HF
: high-frequency

PSI
: peripherally-synapsing interneuron

top1
: DNA topoisomerase I enzyme

TUNEL
: terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling

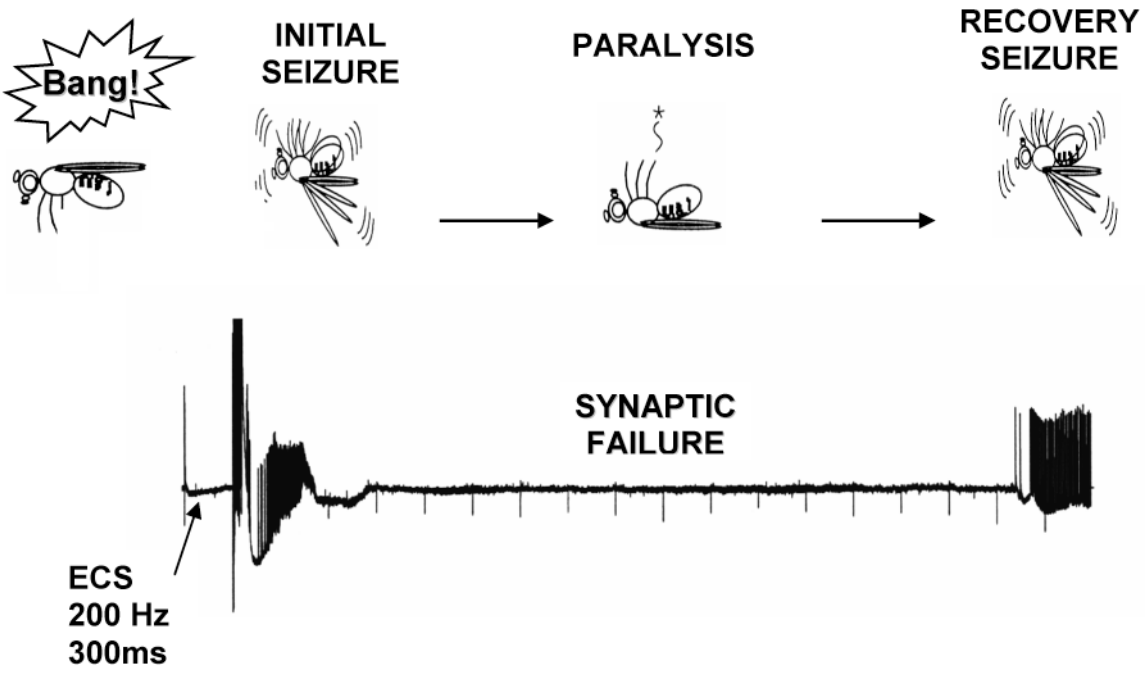

Figure 1. Behavioral and electrophysiological responses of BS mutants to mechanical and electrical stimulation

BS flies exhibit a unique behavioral repertoire following a mechanical stimulation ("bang"), such as a tap on the bench-top and vortex on the vortex mixer: they undergo seizure-like behavioral activity (hyperactivity) and subsequent paralysis. The seizure-like behavior is manifested as intense abnormal contraction, wing-flapping, proboscis extension, and leg-shaking; the paralysis is the cessation of physical activity. Upon recovery from paralysis, flies undergo additional bouts of spontaneous seizure-like behavioral activity that vary in intensity depending on the genotype of the fly. The entire cycle from the initial seizure-like behavior to the restoration of normal behavior is termed as the recovery time. This complex behavioral response can be mimicked on an electrophysiological level by administration of a high-frequency ECS (electroconvulsive shock) to the brain of the fly, which results in seizure-like activity followed by synaptic failure. As the fly recovers, a second bout of seizure-like activity is typically seen. Following ECS-induced seizure-like activity, flies exhibit transiently elevated seizure thresholds, corresponding to the behavioral refractory period.

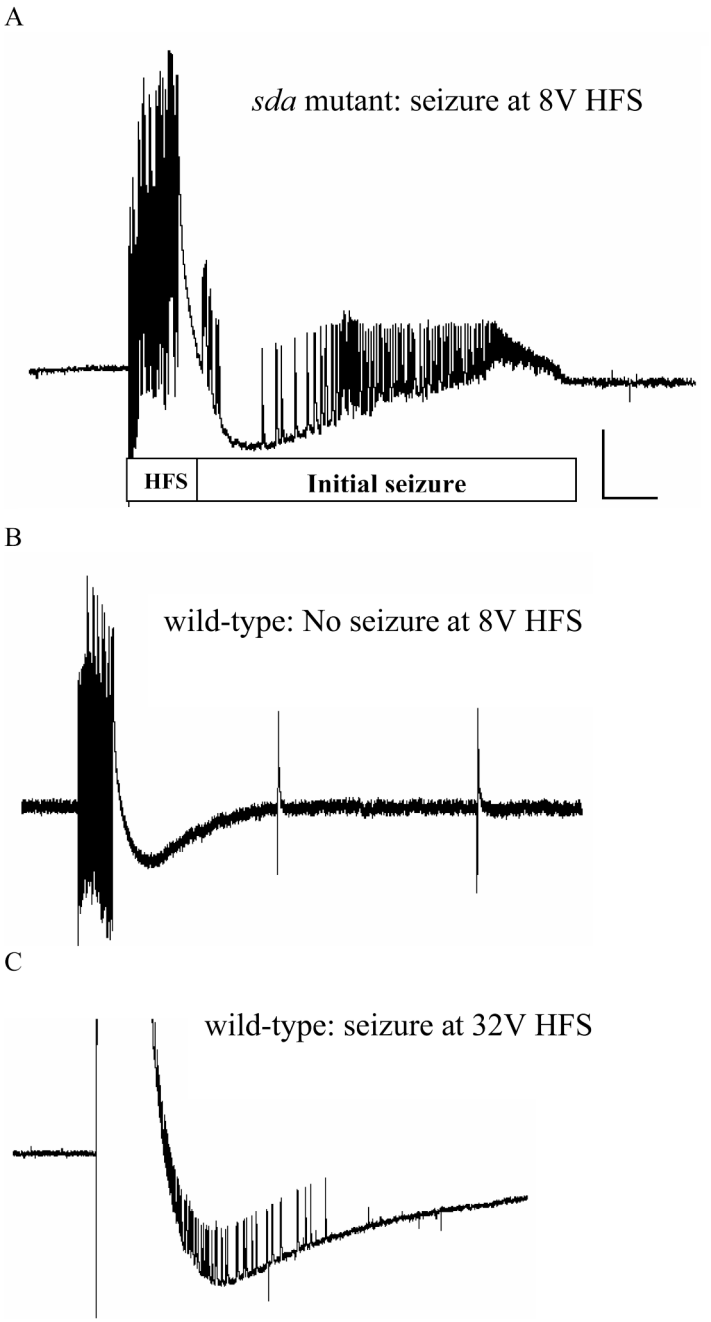

Figure 2. Seizure-like activity in intact *sda* and wild type CS flies

The BS mutant *sda* fly is more susceptible to seizure-like activity than the wild type CS fly and therefore has a much lower seizure threshold. (A) Seizure-like activity is elicited in a *sda* fly by a high frequency stimulus of low strength (8 V) and displayed at a high sweep speed. The HF stimulus (labeled HF) is a short wavetrain (0.5 ms pulses at 200 Hz for 300 ms) of electrical stimuli delivered to the brain. Recording is from a muscle fiber (DLM, dorsal longitudinal muscle) and reflects the activity of the single DLM motoneuron that innervates it. The seizure-like activity is widespread as similar firing can be found in recordings from seven different muscle groups in the fly following HF stimulation (Kuebler and Tanouye, 2000). (B) A low-voltage HF stimulus of 8 V fails to elicit seizure-like activity in a wild type

CS fly because the stimulus is below the seizure threshold. Following the HF stimulus artifact, there is no seizure-like activity observed in this recording displayed at a high sweep speed. Note also that there is no period of synaptic failure and single-pulse stimulation of the GF (0.5 Hz) continues to evoke DLM potentials. Two such effective single-pulse stimuli are depicted in this trace; each was effective in evoking a DLM potential. (C) Seizure-like activity is elicited in a wild type CS fly by a high voltage HF stimulus (32 V) which is above the threshold for seizure. Vertical calibration bar is 20 mV, horizontal calibration bar is 300 ms (figure modified from Kuebler and Tanouye, 2002, figure 1).

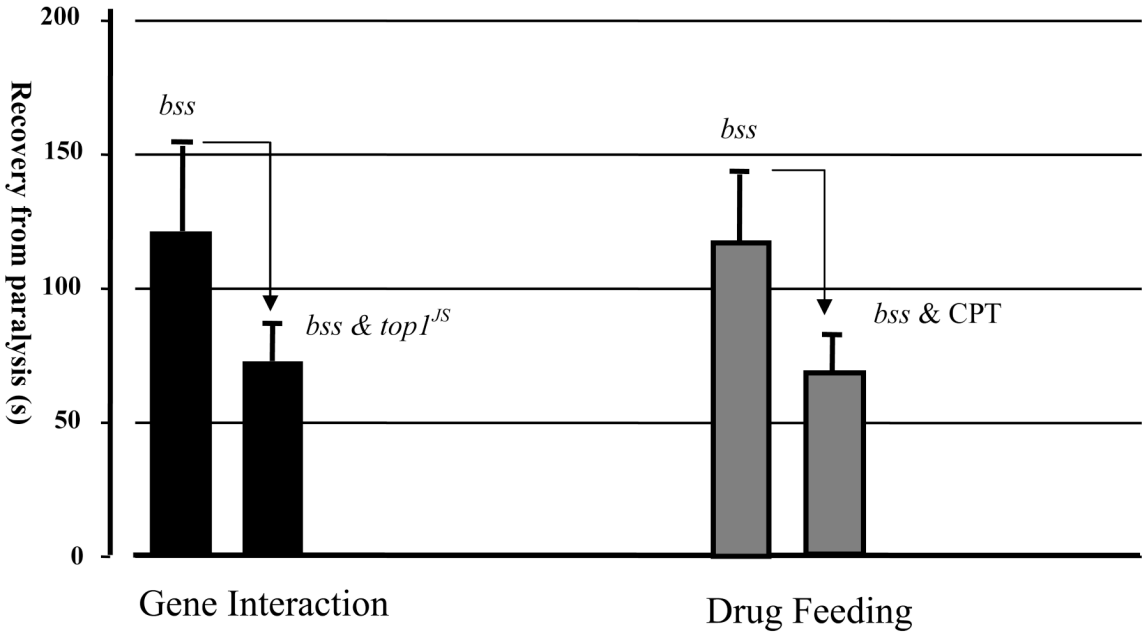

Figure 3. The comparison between genetic interaction and drug feeding

Drug treatment by CPT phenocopies the genetic interaction between *bss* and *top1*^{JS} in reduction of the recovery time. The average recovery time of *bss* is around 120s (3dp), it is reduced to around 70 s in the double mutant *top1*^{JS} *bss*, and 60 s in the CPT-treated *bss* mutant.

Table 1

List of seizure-sensitive mutants and their gene products

Seizure-sensitive Mutant	Gene Product
bang senseless (bss1, bss2)	N/A
bang sensitive (bas1, bas2)	N/A
easily shocked (eas)	ethanolamine kinase (Pavlidis et al., 1994)
knockdown (kdn)	citrate synthase (Fergestad et al., 2006)
slamdance (sda)	aminopeptidase N (Zhang et al., 2002)
technical knockout (tko)	mitochondrial ribosomal protein S12 (Royden et al., 1987)
couch potato (cpo)	RNA-binding protein (Glasscock et al., 2005)
kazachoc (kcc)	K^+ Cl^- cotransporter (Hekmat-Scafe et al., 2006)
stress-sensitive (sesB)	adenine nucleotide translocase (Zhang et al., 1999a)
jitterbug (jbug)	N/A
rock-n-roll (rnr)	N/A

Currently ten different BS mutants have been isolated. The first six mutants comprise the canonical BS strains, characterized by low seizure thresholds (<10 V). The last five BS mutants are non-canonical and exhibit slightly higher seizure thresholds (11-16 V) than the canonical strains.

Table 2

List of seizure-suppressor mutants and their gene products

Seizure-suppressor Mutant	Gene Product
paralytic (*para*^{ST76}, *para*^{JS})	Sodium channel
maleless no-action-potential temperature sensitive (*mle*^{napts})	RNA helicase like protein
Shaker (*sh*)	Potassium channel
Shaking B (*shakB*^{2})	Gap junction protein
escargot (*esg*)	Zinc-figure transcription factor
meiosis defect (*mei-p26*^{EG})	Ring finger B-box coiled-coil-NHL protein
top1(*top1*^{JS})	DNA topoisomerase I