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REVIEW

Drosophila seizure disorders: genetic suppression of seizure susceptibility

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Abstract **Various Drosophila models of human disease have recently received increased interest. The main goal is to uncover the fundamental biological basis for human pathology taking advantage of the power of Drosophila genetics. This review examines a set of Drosophila seizure-sensitive mutations that model human seizure disorders, especially epilepsy. Also described is a novel set of mutations that act as seizure-suppressors that ameliorate epilepsy phenotypes in double mutant combinations.**

Keywords Drosophila, epilepsy, seizure disorders, sodium channel, seizure-suppressor genes

Introduction

Drosophila mutants provide an attractive approach for modeling the genetics of human pathology (Hariharan and Haber, 2003). Models for several disorders have been presented including neurodegeneration, glioma, sleep disorder and Parkinson's disease (Reiter and Bier, 2001; Hirth, 2010; Read, 2011; Sehgal and Mignot, 2011; Guo, 2012; Freeman et al., 2013). Drosophila investigations are especially valuable for defining fundamentally important biological principles underlying disease. Identification of disease-causing genes is also attractive in providing prospects for developing novel therapeutics and the development of high-throughput drug screening platforms. Drosophila modeling also allows the use of genetic interactions. This type of analysis is a powerful way to approach disorders with complex phenotypes: contributions from different genes may be identified, and relationships determined and parsed out. Enhancement and suppression are the two major types of genetic interaction. In this review, we examine Drosophila seizure disorders as a model for human epilepsy. We focus especially on seizure-sensitive mutants, and the modulation of phenotypes by seizure-enhancer and seizure-suppressor mutations. We describe several mutations that appear to suppress seizures by affecting different neural signaling mechanisms.

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Seizure studies in Drosophila

Drosophila seems an unusual organism choice for seizure studies. Compared to human, rat, and mouse epilepsy model systems, there are prominent differences in Drosophila central nervous system (CNS), especially in size and structure. Similar to other invertebrate nervous systems, the fruit fly CNS has a ganglionic structure. The fly brain (supra-esophageal ganglion) is a collection of synaptic neuropiles (Bullock and Horridge, 1965; Rein et al., 2002), unlike the mammalian brain with its layered organization. The adult thoracic ganglion of the fly is a fused compound ganglion organized into several neuromere neuropiles, unlike the mammalian spinal cord. Despite differences in CNS structure, there are similarities between fly and mammalian nervous systems such as in excitable membranes and signaling molecules. Voltage-gated channels, Na^+, K^+, and Ca^{2+} channels are homologous; as are ligand-gated channels, acetylcholine (ACh), glutamate and gamma aminobutyric acid (GABA) receptors. Many human seizure disorders are

caused by channelopathies and defects in these and other signaling molecules can be modeled especially well by Drosophila.

Seizure-like CNS spiking activity can be evoked in Drosophila by electrical stimuli of sufficient intensity delivered to the brain (Fig. 1; Pavlidis and Tanouye, 1995; Kuebler and Tanouye, 2000; Lee and Wu, 2002, 2006). In many aspects, seizure-like activity in the fly resembles seizures in mammals, including humans. Investigations in Drosophila have shown that: a) Drosophila seizure-like activity is extensive, with all fly neurons thus far examined (about 30 neurons) participating in the seizure; b) seizure-like activity manifests as uncontrolled, abnormal neuronal firing that approaches 100 Hz for 3 s; c) every fly has a characteristic seizure threshold (Pisani et al., 2002; Oh and Bainbridge, 2012); d) mutations can modulate seizure-susceptibility with all flies of the same genotype displaying a similar seizure threshold (McNamara, 1994; Noebels, 1996); e) electroconvulsive shock treatment (ECT) raises the threshold for subsequent seizures (Sackeim et al., 1987; Griesemer et al., 1997; Regenold et al., 1998; Lunde et al., 2006), termed refractory period in flies; f) seizures spread along characteristic pathways in the fly CNS that depend on functional synaptic connections (Jacobs et al., 2008; McIntyre and Gilby, 2008; Stefan and Lopes da Silva, 2013; Kroll et al., 2015); g) seizure phenotypes in the fly respond to human antiepileptic drugs such as potassium bromide, valproate, gabapentin, and phenytoin; and h) mutations in several human and fly homologs cause seizures (Table 2).

High Frequency Stimulation (HFS; 0.4 ms pulses at 200 Hz for 300 ms) for evoking seizures is delivered by metal microelectrodes, typically to the brain, but in some studies to the thoracic ganglion (Pavlidis and Tanouye, 1995; Kuebler and Tanouye, 2000; Lee and Wu, 2002; 2006). Seizure-susceptibility may be quantified according to the intensity of the stimulus (Fig. 1, Table 1). Wild type Canton Special flies have a seizure threshold of about 30V HFS (Kuebler et al., 2001). Seizure-sensitive mutants have seizure thresholds with HFS voltages lower than wild type, as low as 2V HFS, in some instances. Seizure-resistant mutants have thresholds higher than wild type, greater than 100V HFS, in some instances.

Seizure-sensitive mutants

Bang-sensitive paralytic mutants

Drosophila seizure-sensitive mutants were discovered while

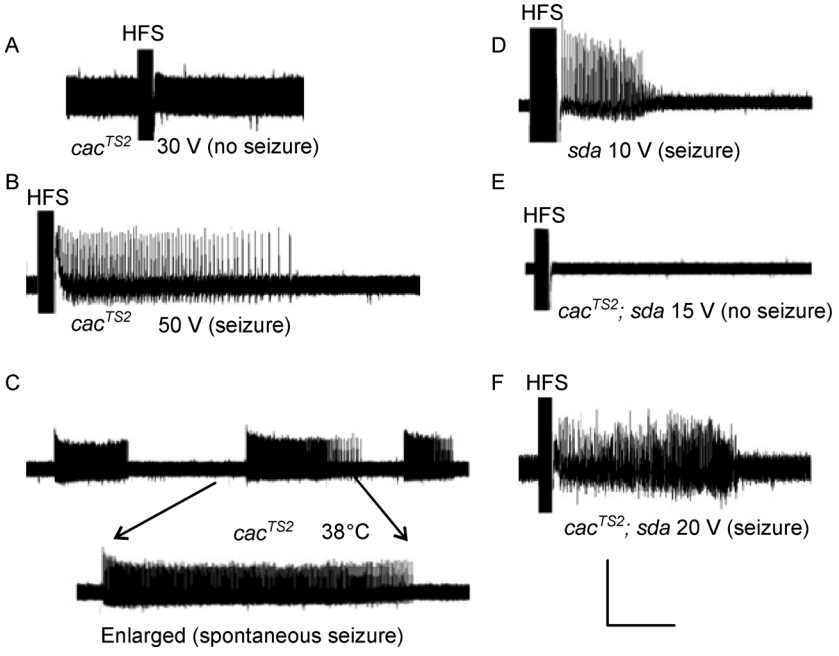

Figure 1 legend. *Drosophila* cac^{TS2} electrophysiology. (A) Electrical recording from a cac^{TS2} DLM fiber showing that seizure-like activity is not evoked by stimulation at 30V HFS, near the wild-type range. (B) Seizure-like activity is observed in cac^{TS2} at a larger stimulus voltage of 50V HFS, indicating that the mutant is seizure-resistant. (C) Spontaneous seizure-like activity observed in cac^{TS2} when the temperature is increased to 38°, indicating that the mutant is seizure-sensitive at restrictive temperatures. Recording shows a representative example of three spontaneous seizure-like discharges. Enlargement (lower trace) shows one of the spontaneous discharges at a higher sweep speed. (D) Recording from a *sda* DLM fiber showing seizure-like activity evoked by a 10V HFS stimulus. (E) Recording from a cac^{TS2}*; sda* DLM fiber showing that a 15V HFS stimulation does not evoke seizure-like activity at this voltage; the double mutant shows a higher threshold indicating seizure-suppression by cac^{TS2}. (F) Recording from a cac^{TS2}*; sda* DLM fiber showing that seizure-like activity is evoked at 20V HFS. Horizontal calibration: 300 msec for C (upper trace); 150 msec. for A-B, C (inset), D-F; Vertical calibration: 20mV.

Table 1 Drosophila seizure-sensitive mutants and their gene products

Seizure-sensitive mutant	Threshold (V HFS)	Gene product	Reference
paralyzed (para^{bss1}, para^{bss2})	3.2, 3.7	Na^+ channel	1
paralyzed (para^{GEFS+})	N/A	Na^+ channel	2
paralyzed (para^{DS})	N/A	Na^+ channel	3
easily shocked (eas^{PC80})	3.4	Ethanolamine kinase	4
slamdance (sda)	6.7	Aminopeptidase N	5
bang sensitive (bas^1, bas^2)	7.6, 3.8	Unknown	6
prickle (pk^{sple})	N/A	LIM domain protein	7
technical knockout (tko^{25t})	9.9	Mitochondrial riboprotein	8
kazachoc (kcc^{DHS1})	17.0	K^+, Cl^- cotransporter	9
couch potato (cpo^{EG1})	11.1	RNA binding protein	10
knockdown (kdn)	20.2	Mitochondrial citrate synthase	11
stress-sensitive (sesB)	N/A	Mitochondrial ATP translocase	12
Focal adhesion kinase (Fak56^{CG1})	N/A	Protein tyrosine kinase	13
shibire (shi^{ts1})	N/A	Dynamin	14, 15, 16
cacophony (cac^{TS2}, cac^{NT27})	N/A	Ca^{2+} channel	17, 18, 19
jitterbug (jbug)	10.5	Unknown	
rock-n-roll (rnr)	N/A	Unknown	

Table lists many mutants identified in Drosophila that cause seizure-sensitivity and their gene products and seizure threshold, when known. Seizure threshold is the voltage of high-frequency stimulation (V HFS) required to evoke seizure-like firing in the DLM. For comparison, the seizure threshold for Canton-Special wild-type flies is 30.1 V HFS (Kuebler et al., 2001). References: 1. Parker et al., 2011; 2. Sun et al., 2012; 3. Schutte et al., 2014; 4. Pavlidis et al., 1994; 5. Zhang et al., 2002; 6. Song and Tanouye, 2006; 7. Tao et al., 2011; 8. Royden et al., 1987; 9. Hekmat-Scafe et al., 2006; 10. Glasscock and Tanouye, 2005; 11. Fergestad et al., 2006; 12. Zhang et al., 1999; 13. Ueda et al., 2008; 14. Salkoff and Kelly, 1978; 15. van der Bliek and Meyerowitz, 1991; 16. Kroll et al., 2015; 17. Smith et al., 1996; 18. Rieckhof et al., 2003; 19. Saras and Tanouye, 2016.

Table 2 Human epilepsy genes causing seizure phenotypes in homologous or similar fly genes

Human gene	Protein	Epilepsy	Fly mutation	Reference
Homologous genes				
SCN1A	Na^+ channel	Generalized epilepsy febrile seizure plus	*para^{bss1},para^{GEFS+}, para^{DS}*	1–5
Prickle1	Planar cell polarity regulator	Progressive myoclonus epilepsy	*prickle*	6–7
SLC12A5 (KCC2)	K^+Cl^- co-transporter	Epilepsy of infancy with migrating focal seizures	*kcc*	8–9
CACNA1A	Ca^+ channel	Childhood spike-wave absence epilepsy	*cac^{TS2}*	10–13
Similar gene functions				
MTTK	Mitochondrial tRNA	Myoclonic epilepsy ragged red fiber disease	*tko^{25t}, sesB, kdn*	15–17

Table lists human epilepsy genes and homologous Drosophila genes that cause seizure phenotypes when mutant. References: 1. Mulley et al., 2005; 2. Lossin, 2009; 3. Parker et al., 2011; 4. Sun et al., 2012; 5. Schutte et al., 2014; 6. Bassuk et al., 2008; 7. Tao et al., 2011; 8. Stödberg et al., 2015; 9. Hekmat-Scafe et al., 2006; 10. Imbrici et al., 2004; 11. Smith et al., 1996; 12. Rieckhof et al., 2003; 13. Saras and Tanouye, 2016; 14. Di Mauro et al., 2002; 15. Royden et al., 1987; 16. Zhang et al., 1999; 17. Fergestad et al., 2006.

characterizing behavioral mutants, in particular, Bang-sensitive (BS) paralytics (Benzer, 1971; Ganetzky and Wu, 1982a, 1982b; Engel and Wu, 1994). BS paralytic mutants are so named because of their behavioral phenotype: in response to a mechanical stress stimulus (a "bang"), these mutants show seizure-like behavior, transitioning into behavioral paralysis. The paralytic period is followed by another bout of seizure-like behavior called a "recovery seizure," after which the BS fly awakens and resumes normal behaviors. Initial and recovery seizures manifest as abnormal uncoordinated motor activity with flapping of wings, shaking of legs, and contracting of abdominal muscles (Benzer, 1971; Pavlidis et al., 1994; Parker et al., 2011). Seizure-like behaviors are correlated with intense seizure-like neuronal firing throughout the CNS (Pavlidis and Tanouye, 1995; Kuebler and Tanouye, 2000). During the beginning of the paralysis period, flies of all genotypes are completely quiescent. Electrophysiological recordings suggest that this paralytic period is due to synaptic failure at many CNS synapses (Pavlidis and Tanouye, 1995; Kuebler and Tanouye, 2000). The duration of the paralytic period varies by genotype, age, and temperature. The BS mutant with the

shortest paralysis duration is *slamdance* (*sda)*, about 30 s. In contrast, *paralyzed*^{bss1} *(para*^{bss1}*)* has a paralytic period lasting as long as 3-4 min with initial complete quiescence, followed by multiple cycles of seizure and quiescence that resemble human tonic–clonic activity (Parker et al., 2011). Several mutations that cause seizure sensitivity in flies are similar to those responsible for some human epilepsies. For example, mutations of the voltage-gated Na^+ channel gene are responsible for seizure-sensitivity in flies and in humans. Numerous mutations of the SCN1A human Na^+ channel gene cause epilepsy, especially generalized epilepsy febrile seizures plus (GEFS +) and Dravet's Syndrome (Mulley et al., 2005; Lossin, 2009). Several mutations of the *para* Na^+ channel gene (*para*^{bss1}*, para*^{bss2}*, para*$^{GEFS+}$*, para*^{DS}) cause seizure-sensitivity in Drosophila (Parker et al., 2011; Sun et al., 2012; Schutte et al., 2014). Mutations in the human homolog of the Drosophila prickle gene cause seizure-sensitivity in flies and progressive myoclonus epilepsy-ataxia in humans (Bassuk et al., 2008; Tao et al., 2011).

Electrophysiology of BS mutants

BS mutants have low seizure thresholds indicating that they are especially sensitive to evoked seizures (Fig. 1, Table 1). For example, the seizure threshold for *sda* mutants is 6V HFS (Zhang et al., 2002). The BS paralytic class is extensive, composed of 20 mutant alleles representing 15 genes and a diverse variety of gene products (Table 1). As one example, the *para*^{bss1} mutation is described here in more detail. Flies carrying *para*^{bss1} have a defect in the voltage-gated Na^+ channel gene (Parker et al., 2011). Voltage-gated Na^+ channels in Drosophila are encoded by *para*, a single large gene spanning about 60 kb of genomic DNA and alternatively spliced to give as many as 57 unique polypeptides (Loughney et al., 1989; Ramaswami and Tanouye, 1989; Thackeray and Ganetzky, 1994; 1995; Feng et al., 1995; O'Dowd et al., 1995; Warmke et al., 1997; Dong, 2007; Olson et al., 2008; Lin et al., 2009; Lin and Baines, 2014). This differs from human nerve and muscle that express functionally distinct voltage-gated Na^+ channels, with diversity arising from the differential expression of 9 different genes (Goldin, 2001; Catterall et al., 2003; Catterall, 2014).

In Drosophila, numerous Na^+ channel mutations have been identified for *para* with 117 reported alleles, including alleles that cause lethality, temperature-sensitive paralysis, olfactory defects and insecticide resistance (Fahmy and Fahmy, 1960; Suzuki et al., 1971; Siddiqi and Benzer, 1976; Wu and Ganetzky, 1980; Ganetzky, 1984; Loughney et al., 1989; Lilly and Carlson, 1990; Pittendrigh et al., 1997). Four mutations have been described that cause seizure-sensitivity: *para*^{bss1}, *para*^{bss2}, *para*$^{GEFS+}$, and *para*^{DS} (Parker et al., 2011; Sun et al., 2012; Schutte et al., 2014). A mis-sense amino acid substitution (L1699F) is responsible for *para*^{bss1} phenotypes and behaves as a gain-of-function mutation (Parker et al., 2011). This mutation generates an abnormal Na^+ channel polypeptide that does not inactivate properly during the action potential causing increased neuronal excitability (Parker et al., 2011). Heterologous voltage clamp analysis in *Xenopus* oocytes indicates that the inactivation defect is due to a depolarizing shift of the voltage dependence of channel inactivation (Parker et al., 2011). Interestingly, a similar depolarizing shift of Na^+ channel inactivation has been described for the human SCN2A channel which is responsible for Neonatal Epilepsy with Late-onset Episodic Ataxia (Schwarz et al., 2016). The *para*^{bss1} mutant is the most severely seizure-sensitive mutant thus far identified in Drosophila. Its seizure threshold is the lowest of all the seizure-sensitive mutants, 3V HFS, a 90% reduction compared to the wild type threshold (Kuebler and Tanouye, 2000; Zhang et al., 2002; Parker et al., 2011). Also, among BS mutants, *para*^{bss1} manifests the longest and most penetrant paralytic behavior. The phenotypes of *para*^{bss1} are difficult to suppress by antiepileptic drug or seizure-suppressor mutation. Parker *et al*. (2011) have presented the mutant as a model for human intractable epilepsy.

Cation-chloride cotransporter knockdown in neurons or in glia causes seizures

Glial cells have been proposed to be contributors to seizure disorders because of their role in maintaining nervous system ionic homeostasis. (Chvatal and Sykova, 2000; D'Ambrosio, 2004; Ueda et al., 2008; Seifert et al., 2010; Devinsky et al., 2013). Compared to dysfunctions in neuronal and synaptic signaling mechanisms, however, glial contributions to seizure have been poorly studied. Studies of Drosophila *kazachoc (kcc)* mutations have shown that these flies are more seizure-sensitive than wild type flies (Hekmat-Scafe et al., 2006; 2010; Rusan et al., 2014). The Drosophila *kcc* gene encodes a K^+-Cl^- cotransporter, homologous to mammalian KCC2, which extrudes K^+ and Cl^- ions from the cell in several different cell types (Mount et al., 1998; Hebert et al., 2004). The mutant defects responsible for causing seizures in Drosophila can occur in either neurons or in glia, with different mechanisms responsible for seizures in the two different cell types (Hekmat-Scafe et al., 2006; 2010; Rusan et al., 2014).

In neurons, the seizure sensitivity of *kcc* mutants is mediated by GABAA receptors, linking kcc seizures with dysfunction of the GABAergic inhibitory system (Hekmat-Scafe et al., 2006, 2010). During a critical period in the developing mammalian brain, there is a major switch in the nature of GABAergic synaptic transmission (Ben-Ari, 2002; Ben-Ari et al., 2007). The pattern of GABAergic transmission in the neonatal is depolarizing and excitatory; this transmission switches developmentally to hyperpolarizing and inhibitory, the pattern of the mature brain. This switch is believed to be important in shaping activity-dependent mechanisms for determining neuronal connectivity. The GABAergic developmental switch may be particularly

vulnerable to dysfunction leading to seizure disorders. The developmental GABA switch is mediated primarily by KCC2 determining the intracellular concentration of Cl^- and, hence, the reversal potential for GABA. The seizure phenotype of *kcc* mutants was shown to be due to a developmental switch in Drosophila GABAergic signaling (Hekmat-Scafe et al., 2006). The kcc protein is widely expressed in brain neuropil, and its level rises during development. Young *kcc* mutant flies with low kcc levels are seizure-sensitive, and this sensitivity disappears with age. Sensitivity to seizures decreases steadily for the first 2-3d of adulthood and then falls precipitously 1d later (Hekmat-Scafe et al., 2006). Genetic and pharmacological experiments indicate that *kcc* seizure sensitivity occurs via excitatory GABAergic signaling and, in particular, the kcc GABA switch. This mechanism appears similar to seizures due to reduced KCC2 function in mouse and humans (Hubner et al., 2001; Woo et al., 2002; Boettger et al., 2003; Tornberg et al., 2005).

Rusan *et al*. (2014) used RNAi to knockdown *kcc* selectively from either neurons or glia, or from both cell types together. In each case, loss of *kcc* caused seizure-sensitivity. For neurons, *kcc* loss appears to cause seizures by reducing inhibitory $GABA_A$ currents, however, glial loss of *kcc* appears to cause seizures by a different mechanism. Reduced *kcc* in glia causes nerve cell swelling and blood-brain barrier degradation due to ionic homeostasis dysfunction (Rusan et al., 2014). Results indicate that the ionic homeostasis dysfunction is mainly excess K^+ accumulation because of a failure of glial cells to adequately buffer extracellular K^+ during sustained action potential activity. Increases in $[K]_o$ during seizures is well documented and has been suggested to play a causal role in the formation of epileptic foci through a positive feedback loop (Kandel and Spencer, 1961; Fertziger and Ranck 1970; Zuckermann and Glaser, 1970). A rise in $[K]_o$ shifts E_k in a positive direction. Resting membrane potential is largely determined by E_k and hence, an increase in E_k depolarizes the membrane leading to increased excitability (Somjen 2004). Computional studies of neuron models in which $[K]_o$ is treated as a bifurcation parameter reveals transitions from quiescence to tonic firing, bursting, and eventual depolarization block, for increasing $[K]_o$ (Kager et al., 2000; Cressman et al., 2009; Florence et al., 2009; Barreto and Cressman, 2011).

Some temperature-sensitive paralytic mutants are seizure-sensitive

Temperature-sensitive (TS) paralytic mutants show normal behavior at "permissive" temperatures, usually room temperature. These mutants are paralyzed at "restrictive" temperatures, usually high temperature. Two TS paralytic mutants are seizure-sensitive mutants *shibire (shi)* and *cacophony (cac).* The *shi* gene encodes Dynamin, a GTPase required in chemical synaptic transmission for endocytosis and vesicle recycling (van der Bliek and Meyerowitz, 1991). In the shi^{ts1} mutant, seizure-like phenotypes of behavioral hyperexcitability and spontaneous seizure-like neuronal spiking are observed transiently following a shift from permissive room temperature to 29°C restrictive temperature (Salkoff and Kelly, 1978; Kroll et al., 2015). These are followed by paralysis and a loss of chemical synaptic transmission, phenotypes due to synaptic vesicle depletion (Grigliatti et al., 1973; Siddiqi and Benzer, 1976; Koenig and Ikeda, 1989; van der Bliek and Meyerowitz, 1991). Similar findings are observed for *cac* that encodes the N-type Ca^{2+} channel responsible for presynaptic neurotransmitter release (Fig. 1; Smith et al., 1996; Kawasaki et al., 2000; Kuromi et al., 2004). In the cac^{TS2} mutant, seizure-like phenotypes of behavioral hyperexcitability and spontaneous seizure-like neuronal spiking are observed transiently following a shift from permissive temperature to the restrictive temperature of 38°C (Rieckhof et al., 2003; Saras and Tanouye, 2016). These transient seizure-like phenotypes are subsequently replaced by behavioral paralysis and loss of chemical synaptic transmission phenotypes (Kawasaki et al., 2000; Kuromi et al., 2004).

Seizure-suppressor mutations

Seizure-suppressors interact genetically with BS mutations to ameliorate seizure phenotypes. Suppressors are identified in double mutant combinations to reveal genetic interaction. A BS mutation, for example, *eas* or $para^{bss1}$ is used to create a seizure-sensitive genetic background. The double mutant is then constructed with the putative suppressor. Suppression manifests in the double mutant if BS phenotypes are ameliorated to become more like the wild type. That is, BS seizure-like phenotypes are suppressed (Fig. 1). A collection of 28 seizure-suppressor mutations in 16 genes has been identified (Table 3). An unexpected finding from studying seizure-suppressor mutations is their apparent abundance and ease of identification. The large number of identified mutations suggests that seizure-suppression can occur by numerous different mechanisms. Some suppressors identify gene products not previously associated with neuronal signaling leaving speculation about a suppression mechanism, such as *fat facets*, apparently relying on a de-ubiquitination mechanism (Paemka et al., 2015). Some suppressors encode well-studied gene products involved with nervous system function that have allowed us to consider five likely mechanisms for how seizure-like activity might be suppressed by second-site mutations.

Suppressing seizures through opposing effects on nerve excitability

The properly functioning nervous system is a balance of excitation and inhibition. Seizures are due to an imbalance: an excess of excitation or too little inhibition. The $para^{ST76}$,

Table 3 Seizure-suppressor mutants and their gene products

Seizure-suppressor mutant	Gene product	Reference
paralyzed (*para^{ST76}*, *para^{JS1}*)	Na channel	1, 2
male lethal (*mle^{napts}*)	Na channel regulator	1
shakingB (*shakB^{2}*, (*shakB^{JS}*)	Gap junction channel	2, 3
Shaker (*Sh^{KS133}*)	K channel	1
escargot (*esg^{EP684}* + 4 alleles)	Zn-finger transcription factor	4
snail (*UAS-sna#61*)	Zn-finger transcription factor	4
kazal-domain protein-1 (*kdp1*)	Kazal-type serine protease inhibitor	4
kazal-domain protein-2 (*kdp2*)	Kazal-type serine protease	4
meiosis-P26 (*mei-P26^{EG16}*, *mei-P26^{1}*)	Ring finger B-box coiled-coil-NHL protein	5
suppressor of eas7 (*su(eas7)*)	Unknown (Glasscock et al., 2005)	5
suppressor of eas13 (*su(eas13)*)	Unknown (Glasscock et al., 2005)	5
topoisomerase I (*top1^{JS}* + 3 alleles)	DNA topoisomerase type I	6
fat facets (*faf^{B3}*, *faf^{BX3}*, *faf^{BX4}*)	Deubiquitinating enzyme	7
gilgamesh (*gish^{04895}*)	Casein kinase	8
shibire (*shi^{ts1}*, *shi^{ts2}*)	Dynamin	9
cacophony (*cac^{TS2}*)	Ca^{2+} channel	10

Table lists many mutants that suppress seizure-sensitivity in double mutant combinations with bang-sensitive paralytic seizure-sensitive mutants. Included are the gene products, when known. References: 1. Kuebler et al., 2001; 2. Song and Tanouye, 2007; 3. Song and Tanouye, 2006; 4. Hekmat-Scafe et al., 2005; 5. Glasscock et al., 2005; 6. Song et al., 2007; 7. Paemka et al., 2015; 8. Howlett et al., 2013; 9. Kroll et al., 2015; 10. Saras and Tanouye, 2016.

para^{JS} and *mle^{napts}* mutations reduce nerve excitability by voltage-gated Na^+ channel loss-of-function and act as strong seizure-suppressor mutations (Ganetzky and Wu, 1982a; Kuebler and Tanouye, 2000; Kuebler et al., 2001; Song and Tanouye, 2007). Hypoexcitability from these mutations can suppress seizure-like activity by compensating hyperexcitatibility caused by BS mutations such as *sda* and *eas.*

Suppressing seizures by limiting action potential firing frequency

Seizure-like activity is characterized by uncontrolled high-frequency action potential firing by neurons participating in the seizure. Mutations that disrupt the capacity for high frequency firing can cause seizure-suppression. The K^+ channel mutation *Sh^{KS133}* increases action potential duration, leading to longer refractory periods (Tanouye et al., 1981; Kuebler and Tanouye, 2000; Kuebler et al., 2001). Spikes of neural activity cannot be generated during the refractory period, apparently leading to seizure-suppression by limiting axons to low action potential firing frequencies. Thus, despite *Sh^{KS133}* generally causing nervous system hyperexcitability, the mutation is also a seizure-suppressor because the high-frequency action potential firing required for seizure-like activity is not supported by axons in these mutants.

Suppressing seizures by preventing synchronized firing

Seizure-like activity is the uncontrolled, synchronous firing of populations of neurons. In the Drosophila nervous system, synchronous firing is mediated by electrical synaptic transmission. The *ShakB^{2}*, a mutation of the gap junction innexin channel, causes a defect in electrical synapses (Phelan et al., 1996; 1998; Phelan and Starich, 2001). Seizure-like activity is suppressed because *ShakB^{2}* limits electrical synaptic transmission, which appears to be critical for synchronizing the activity of populations of firing neurons and for the spread of seizure-like excitation (Kuebler et al., 2001; Song and Tanouye, 2006).

Suppressing seizures by neuronal cell death

Seizure-suppression has been identified for the Type I DNA topoisomerase mutation *top1^{JS}* and subsequently, for the topoisomerase I inhibitor camptothecin (Song et al., 2007; Song et al., 2008). Type I topoisomerases function in DNA replication and transcription to relieve the torsional stress of supercoils by binding to DNA, cleaving it and relaxing the helix (Champoux, 2001). The discovery of the seizure-suppressor *top1^{JS}* was unexpected, because DNA topoisomerases have not been linked to seizures, seizure control, or any other neuronal excitability functions. The Drosophila *top1* gene is essential and mutations are often lethal. However, the *top1^{JS}* mutation is a viable, partial loss-of-function mutation with no obvious neurological phenotypes other than acting as a BS suppressor (Song et al., 2007). Thus, *top1^{JS}* mutant flies are not TS paralytics (hypoexcitable). They show no obvious limitations in action potential firing frequency or ether-induced leg-shaking unlike *Sh^{KS133}* mutants. And the *top1^{JS}* mutant shows no electrophysiological defects indicating abnormal electrical transmission unlike *shakB^{2}*. Instead, seizure-suppression by *top1^{JS}* is due to neuronal cell death in the mutant (Song et al., 2007). Increased cell death is observed in the *top1^{JS}* brain. However, overexpression of

DIAP1, which is an anti-apoptotic protein, rescues neuronal cell death and renders *top1*^{JS} incapable of suppressing seizures (Song et al., 2007). As reported, *DIAP1* rescue occurs when driven in all neurons using the pan-neuronal GAL4-driver, ELAV-GAL4. Rescue with cell-type specific GAL4-drivers has not been reported.

Suppressing seizures by adjusting functional neurocircuitry

Molecules responsible for chemical synaptic transmission are a potentially rich source for identifying seizure-suppressor mutations. Suppression is expected especially for mutations that boost synaptic inhibition, such as molecules involved in GABAergic signaling. These are also expected for mutations that diminish synaptic excitation, such as molecules involved in cholinergic transmission. Thus far, none of these types of suppressors have been identified. Instead, *shi*^{ts1} and *cac*^{TS2}, that are involved in general synaptic transmission have been identified as seizure-suppressor mutations. As as single mutants, *shi*^{ts1} and *cac*^{TS2} are both TS seizure-sensitive mutants. Surprisingly, in double mutant combinations with BS mutants, *shi*^{ts1} and *cac*^{TS2}, are each found to additionally act as seizure-suppressors (Fig. 1; Kroll et al., 2015; Saras and Tanouye, 2016). Not only are *shi*^{ts1} and *cac*^{TS2} seizure-suppressors, they are the two most effective suppressors that we have identified. Both can suppress phenotypes in *para*^{bss1}, the most difficult of the BS mutants to suppress (Kroll et al., 2015; Saras and Tanouye, 2016).

We presume that seizure-suppression by *shi*^{ts1} and *cac*^{TS2} work by interfering with chemical synaptic transmission in many or most neurocircuits in the fly. Modest interference in synaptic transmission at room temperature is sufficient to suppress weak BS mutants, such as *sda*. Stronger disabling of synaptic transmission is necessary to suppress the stronger BS *para*^{bss1} . We refer to this as "neurocircuitry" suppression of seizures. Much of this mechanism is speculation because neurocircuitry for the fly is generally not well understood and, in particular, little is known about the circuitry responsible for seizures. Also, *shi*^{ts1} and *cac*^{TS2} are apparently interfering with chemical synaptic transmission in all circuits, although some may not be involved in seizures. Although seizure circuits in the fly are not known, it is convenient to consider neurocircuit suppression with respect to the seizure model of Kuebler et al. (2001; Fig. 2).

a. Trigger circuit. The central feature is a seizure trigger circuit that has a defined threshold for activation (Fig. 2). It is the feature of the trigger circuit that is responsible for the seizure threshold characteristic for each genotype. The location of the trigger circuit is not clear, but may be in the mushroom body (Hekmat-Scafe et al., 2010).

b. Input circuit. These inputs are presynaptic to the trigger circuit. They may be populations of fibers or an extensive neural circuit. The inputs appear to show temporal and spatial summation (Kuebler and Tanouye, 2000). Because of BS behavior, some of these inputs may come from mechanosensory afferents.

c. Output circuit. This circuit delivers the triggered

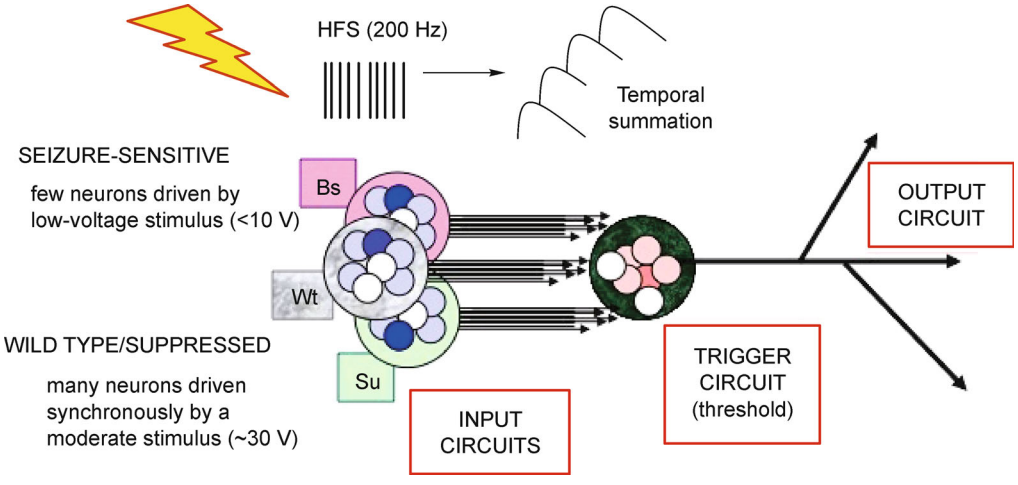

Figure 2 A model for seizure suppression in Drosophila. This model consists of three presynaptic input circuits that act to trigger seizures (labeled Wt, Bs, and Su). These inputs drive a "trigger circuit" that is capable of delivering seizures throughout the Drosophila central nervous system via an "output circuit." It is the threshold of the trigger circuit that determines the seizure threshold for each individual fly. Although the input circuits are given separate names, we have found no qualitative features that distinguish them. In a normal wild type fly, stimulation of two input (say, Bs and Wt) with an HFS electrical stimulus wavetrain triggers a seizure. Synaptic potentials from Bs and Wt summate temporally and spatially in the trigger circuit to generate the seizure. In a BS mutant fly, it is much easier to trigger a seizure and stimulation of only the Bs input is sufficient to bring the trigger circuit to threshold. Many suppressor mutants are also seizure resistant; it is more difficult to trigger the seizure, and necessary to drive all three inputs (Bs, Wt, and Su). In many BS;suppressor double mutant genotypes, seizure threshold has been restored to near the wild type level and a seizure is triggered by stimulating two inputs (say, Bs and Wt). We are presuming that larger stimulation voltages drive greater numbers of input neurons since single cell excitability appears to remain unchanged across different wild type and mutant strains (Kuebler et al., 2001).

seizure throughout the fly central nervous system. Thus far, all known motor outputs appear to participate in the seizure with the possible exception of the tergotrochanter muscle (Pavlidis and Tanouye, 1995; Kuebler and Tanouye, 2000).

Neural circuit analysis of shi^{ts1} suppression indicates that it is due to endocytosis impairment in the output circuit (Kroll et al., 2015). Analysis is facilitated because of the dominant negative functioning of the Shi^{ts1} protein (Kitamoto, 2001; Pfeiffer et al., 2012). Thus, selective impairment of endocytosis can be accomplished by overexpressing shi^{ts1} transgenes using GAL4/UAS. Expression of shi^{ts1} transgenes either in all neurons, in excitatory cholinergic neurons, or in the giant fiber (GF) neuron was sufficient for seizure-suppression (Kroll et al., 2015). From this, it was concluded that suppression is due to the output circuit, and the GF neuron plays an important role in the delivery of seizure-like activity (Kroll et al., 2015). Neural circuit analysis for cac^{TS2} suppression is less straightforward, but Saras and Tanouye (2016) suggest that it is due to impairment of the input circuit. It should be possible to identify circuits responsible for suppression by the use of GAL4/UAS restricted expression of cacRNAi. Surprisingly, expressing cacRNAi selectively in excitatory interneurons (cha-GAL4 driver), or inhibitory interneurons (GAD-GAL4 driver) results in substantially less suppression than using pan-neuronal drivers (Saras and Tanouye, 2016). Suppression is accompanied by an increase in evoked seizure threshold. In the double mutant *BS*; cac^{TS2}, higher HFS voltages are required to evoke seizure-like activity than in single BS mutants (Saras and Tanouye, 2016). This indicates that a larger number of inputs must be driven synchronously to trigger the seizure (Kuebler et al., 2001). This is because the seizure trigger circuit threshold is characteristic for different genotypes. However, in this instance, for cac^{TS2} suppression, it was proposed that input circuits are less effective because of impaired synaptic transmission. Seizures are only evoked when more of these weakened inputs drive the trigger circuit (Saras and Tanouye, 2016).

Drosophila as a model for antiepileptic drug discovery and testing

About three million Americans are afflicted with epilepsy (Kwan and Brodie, 2000; Shneker and Fountain, 2003). Two-thirds of patients respond to antiepileptic drug (AED) treatment, although seizure control is not always complete and there are side effects. For about one million patients with intractable epilepsy, sufferers do not respond at all to currently available AEDs. There continues to be a great need for new AEDs and the biggest appeal of a Drosophila epilepsy model is as a platform for AED discovery. The same features that facilitated the identification of BS mutants and seizure-suppressor mutants in Drosophila are also advantageous for drug testing. Tests for behavioral bang-sensitivity and seizure-like behavior are robust. Electrophysiology tests provide additional characterization and allow quantitative measures of seizure-susceptibility. Flies reproduce rapidly and are small in size allowing easy manipulation and the rapid testing of large populations of subjects.

Preliminary experiments have shown that a number of drugs, including several AEDs, ameliorate the severity of Drosophila BS phenotypes. Some drugs are found to reduce the severity of BS mutant phenotypes, mostly by reducing paralytic recovery time. These include valproate, phenytoin, gabapentin, potassium bromide, and carbenoxolone; but not carbamazepine, ethosuximide and vigabactrin (Kuebler and Tanouye, 2002; Reynolds et al., 2003; Tan et al., 2004; Song and Tanouye, 2006; Song et al., 2008; Howlett and Tanouye, 2013). Three drugs that are Top1 inhibitors, camptothecin, apigenin, and kaempferol, were also found to reduce the $para^{bss1}$ recovery period (Song et al., 2008).

Drug testing Drosophila larvae

Stilwell *et al*. (2006) developed an elegant screen for AEDs in Drosophila larvae. The screen identified drugs that prevent poisoning by picrotoxin (PTX). PTX is a convulsant, and an antagonist for the $GABA_A$ receptor. PTX feeding causes robust seizure-like activity with sustained contractions of the larval body wall and a disruption of locomotion. Lethality (LD_{100}) occurs at 0.5 mg/ml PTX and is rescued by four drugs: phenytoin, nifedipine, flunitrazepam and levetiracetam (Stilwell et al., 2006). Additionally, the drugs ethosuximide, zonisamide, diazepam and lamotrigine gave partial rescue. Rescue was not effective for an additional 14 drugs, including valproate, carbamazepine, topiramate, and gabapentin (Stilwell et al., 2006).

Drug studies using the AED valproate

Efficient drug delivery methods facilitate the identification of drug candidates from libraries of chemical compounds. Most conveniently utilized are feeding methods: flies are starved for a short time and then fed drug in sucrose solution (Tan et al., 2004; Song et al., 2008, for example). Drug feeding is simple and straightforward allowing large numbers of flies to be tested. These are similar to methods used historically for delivering chemical mutagens such as ethylmethanesulfonate (Watanabe and Yamazaki, 1976). Trial experiments evaluating drug delivery and effectiveness in Drosophila have used the AED valproate. Valproate is a broad-spectrum AED that is one of the most widely used drugs for treatment of generalized and partial seizures in human patients. The broad efficacy of valproate appears to be due to effects on multiple molecular targets, especially blockage of voltage-gated Na^+ channels and T-type Ca^{2+} channels; and potentiation of inhibitory GABA responses (Loscher, 2002; White et al., 2007; Landmark, 2008; Greenhill and Jones, 2010).

Valproate has variable effects on Drosophila seizure-

susceptibility. Several experiments have found that feeding valproate to adults or larvae is minimally effective at reducing seizure-like phenotypes (Stilwell et al., 2006; Song et al., 2008). In contrast, direct injection of valproate into the adult brain is effective at reducing seizures (Kuebler and Tanouye, 2002). Some of valproate feeding ineffectiveness is due to chemical detoxification enzymes present in the gut of many insects including Drosophila (Willoughby et al., 2006; Chung et al., 2009). Gut detoxification enzymes can be by-passed by injecting valproate directly into the blood stream (Howlett and Tanouye, 2013). Valproate injection is considerably more effective for seizure-suppression compared with drug feeding (Song et al., 2008; Howlett and Tanouye, 2013). In addition to detoxification mechanisms, valproate feeding ineffectiveness is due to the blood-brain barrier composed of subperineural glia and pleated septate junctions that provide a physical barrier with selective transporters, including the ATP binding transporter Mdr65 (Carlson et al., 2000; Stork et al., 2008; Mayer et al., 2009; Rusan et al., 2014). Utilizing the blood-brain barrier mutations, such as the Mdr65 mutation, appears to further improve valproate injection results under certain circumstances (Howlett and Tanouye, 2013).

Conclusion

Despite the frequency of human epilepsy, in the vast majority of cases a comprehensive understanding of the disease is lacking due to its complexity and heterogeneity. The complicated nature of epilepsy is confirmed by studies on Drosophila seizure-susceptibility. Complexity arises from the large number and diverse nature of mutations modifying seizure-susceptibility. The complexity is furthered by interactions between mutations, for example, the interaction of seizure-sensitive with seizure-suppressor mutations. Even so, Drosophila is an outstanding model for studying seizure disorders with numerous experimental capabilities facilitating its use for studying seizures. Drosophila has a simpler nervous system than humans and it has been well characterized electrophysiologically. Each individual fly has a quantifiable seizure threshold indicating a characteristic seizure-susceptibility. Seizure-susceptibility is modified by mutations readily identified because of robust behavioral and electrophysiological phenotypes. Of the large number of mutations modifying seizure-susceptibility, some are not surprising such as ion channelopathies like voltage-gated Na^+ channel mutations and gap junction channel mutations. Others are unexpected such as prickle, aminopeptidase N, and DNA topoisomerase I mutations. Furthermore, for examining the combinatorial effects of genetic interactions, well-defined genetic backgrounds can be constructed to facilitate experimental analysis. Finally, Drosophila has outstanding potential as a platform for drug discovery. New drug targets can be defined from seizure-suppressor mutations, such as camptothecin, a top1 inhibitor. Drug screening platforms for new AEDs may also be productive as we resolve issues of the blood-brain barrier and detoxification. Thus, the combination of these features make Drosophila a powerful model for human seizure-susceptibility defects with the possibility of developing novel seizure therapeutics.

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Compliance with ethics guidelines

Arunesh Saras, Laura E. Simon, Harlan J. Brawer, Richard E. Price, Mark A. Tanouye declare that they have no conflict of interest.

This article does not contain any studies with human or vertebrate animal subjects performed by any of the authors.

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