Genetic Modifications of Seizure Susceptibility and Expression by Altered Excitability in Drosophila Na+ and K+ Channel Mutants

Jisue Lee and Chun-Fang Wu

Department of Biological Sciences, University of Iowa, Iowa City, Iowa

Submitted 10 May 2006; accepted in final form 6 June 2006

Lee, Jisue and Chun-Fang Wu. Genetic modifications of seizure susceptibility and expression by altered excitability in Drosophila Na+ and K+ channel mutants. J. Neurophysiol. 96: 2465–2478, 2006; doi:10.1152/jn.00499.2006. A seizure-paralysis repertoire characteristic of Drosophila “bang-sensitive” mutants can be evoked electroconvulsively in tethered flies, in which behavioral episodes are associated with synchronized spike discharges in different body parts. Flight muscle DLMs (dorsal longitudinal muscles) display a stereotypic sequence of initial and delayed bouts of discharges (ID and DD), interspersed with giant fiber (GF) pathway failure and followed by a refractory period. We examined how seizure susceptibility and discharge patterns are modified in various K+ and Na+ channel mutants. Decreased numbers of Na+ channels in napΔ flies drastically reduced susceptibility to seizure induction, eliminated ID, and depressed DD spike generation. Mutations of different K+ channels led to differential modifications of the various components in the repertoire. Altered transient K+ currents in Sh133 and Hk mutants promoted ID induction. However, only Sh133 but not Hk mutations increased DD seizure and GF pathway failure durations. Surprisingly, modifications in sustained K+ currents in eag and Shab mutants increased thresholds for DD induction and GF pathway failure. Nevertheless, both eag and Shab, like Sh133, increased DD spike generation and recovery time from GF pathway failure. Interactions between channel mutations with the bang-sensitive mutation bss demonstrated the role of membrane excitability in stress-induced seizure-paralysis behavior. Seizure induction and discharges were suppressed by napΔ in bss nap double mutants, whereas Sh heightened seizure susceptibility in bss Sh133 and bss ShΔ2 double mutants. Our results suggest that individual seizure repertoire components reflect different neural network activities that could be differentially altered by mutations of specific ion channel subunits.

INTRODUCTION

The striking Drosophila bang-sensitive behaviors (Benzer 1973) are highlighted in an interesting category of stress-sensitive mutants that display a characteristic seizure-paralysis phenotype on excessive mechanical stimulation. Although these mutations cause a variety of molecular defects, they all lead to a similar seizure behavioral repertoire (Ganetzky and Wu 1982; Pavlidis et al. 1994; Royden et al. 1987; Zhang et al. 2002). Vertebrate seizure (Fishcer 1989) is also known to involve highly heterogeneous molecular mechanisms (Noebels 2003; Scheffer and Berkovic 2003). Among the various classes of seizure and epilepsy syndromes, several Na+ and K+ channel genes are the prominent identified genetic factors. In addition, several voltage-dependent Na+ and K+ channels are the known targets of antiepileptic drugs (Yogeesswari et al. 2004). A collection of Drosophila Na+ and K+ channel mutations that affects membrane excitability (Singh and Wu 1999; Wu and Ganetzky 1992) offers an opportunity to investigate the roles of molecularly identified channel subtypes in seizure susceptibility and expression. Moreover, the ease of generating double mutants allows an examination of how ion channel mutations modify the functioning of neural circuits in the seizure-prone, bang-sensitive mutants (Ganetzky and Wu 1982).

Seizure and epileptic condition are complex phenomena involving collective activity and interaction among a large number of elements in neural networks. Analyses in simple circuits with identified neural elements thus provide a promising approach to reveal the molecular and physiological bases of seizure phenomena. It has been documented that intense brain stimulation in a tethered Drosophila (Pavlidis and Tanouye 1995) can induce an electroconvulsive behavioral repertoire in parallel with a fixed sequence of physiological events (Lee and Wu 2002) in the giant fiber (GF) pathway that mediates jump-and-flight escape reflex (Engel and Wu 1992; Tanouye and Wyman 1980). The electroconvulsive seizure can be detected along different body axes (Lee and Wu 2002), which begins with a bout of initial discharge (ID) of seizure spikes followed by a suppression of general neural activity and a subsequent bout of delayed discharge (DD). These events correlate with the behavioral sequence of initial muscle spasm, complete paralysis, and delayed spasm before the fly recovers. Similar to some vertebrate epilepsy syndromes, electroconvulsive seizure is followed by a refractory period during which the fly is temporarily resistant to further seizure induction. The physiological events underlying the seizure repertoire are best monitored in the indirect flight muscles’ dorsal longitudinal muscles (DLMs) that display the GF pathway output. Electroconvulsion initiates ID, which is followed by transmission failure of the GF pathway. Recovery of the GF pathway is accompanied by DD seizure spikes (Lee and Wu 2002).

Our study focused on several well-characterized mutants with defects in Na+ and K+ currents. Drosophila K+ channel mutants exhibit leg shaking and a variety of hyperexcitable behaviors (Kaplan and Trout 1969; Singh and Wu 1999; Wu et al. 1983). The structure–function relationships of the channel subunits affected in these Drosophila mutants have been elucidated and their homologous counterparts in vertebrates have been established (Coetzee et al. 1999). The Shaker (Sh) gene, homologous to members of the vertebrate Kv1 family, encodes the α-subunit of a voltage-activated K+ channel (Kamb et al. 1987; Papazian et al. 1987; Pongs et al. 1988) that mediates a...
transient current $I_A$ (Haugland and Wu 1990; Salkoff 1983). Hyperkinetic (Hk) codes for an auxiliary $\beta$-subunit of the Shaker $I_A$ channels (Chouinard et al. 1995; Wang and Wu 1996; Yao and Wu 1999). Shab, the Kv2 homologue, encodes the $\alpha$-subunit of a voltage-activated K$^+$ channel mediating a sustained $I_K$ (Singh and Singh 1999). The products of either $a$ go-go ($eag$) mediate a sustained K$^+$ current in the oocyte expression system (Warmke et al. 1991) and is thought to affect multiple K$^+$ currents in vivo (Zhong and Wu 1991, 1993). We also examined the effect of hypoexcitability caused by a reduced number of Na$^+$ channels in nap$^{ts}$ mutants (Wu et al. 1978). This specific male lethal allele, male$^{nap}$ (hereafter abbreviated as nap$^{ts}$) decreases the expression of paralytic (pnd$^d$), the structural gene for the Na$^+$ channel $\alpha$-subunit (Kernan et al. 1991; Loughney et al. 1989). These voltage-activated Na$^+$ and K$^+$ ion channels have been shown to shape action potentials and regulate spike patterns in Drosophila (Saito et al. 1993; Singh and Wu 1989; Yao and Wu 1999).

A previous study examined the hypothesis of contrasting effects of Na$^+$ and K$^+$ channel mutations on seizure susceptibility on the basis of ID induction (Kuebler et al. 2001). We set out to examine the entire seizure repertoire and analyzed alterations in expression pattern, as well as seizure induction based on DD and GF pathway failure in different ion channel mutants. The results delineate the differential effects of the Na$^+$ and K$^+$ channel subtypes on specific components of the seizure repertoire. In double mutants, the interactions between ion channel mutations with bang-sensitive mutations established the role of membrane excitability in the mechanical stress-induced seizure repertoire typical of the classical bang-sensitive mutants. The specific effects of individual mutations on the repertoire components and the correlations among the altered components in different mutants provide a basis for further genetic dissection of seizure mechanisms in the neural circuits underlying the seizure repertoire. Preliminary accounts of this work previously appeared in abstract form (Lee and Wu 1998, 2001).

**METHODS**

**Fly strains**

*Drosophila melanogaster* strains were kept in 9.5 x 2.5-cm glass vials with standard cornmeal medium and reared at room temperature. Adult flies used for the experiments were 3–12 days old. Wild-type flies were of the strain Canton-Special (CS). More than one mutant allele of each gene was examined to confirm the mutant phenotype, thus minimizing the possibility of unidentified second site effects. All mutant alleles selected for the study here were previously characterized electrophysiologically.

Excitability mutants examined include those defective in Na$^+$ or K$^+$ currents: male lethal$^{nap}$ (nap$^{ts}$) with reduced Na$^+$ current (Kernan et al. 1991; Wu and Ganetzky 1992; Wu et al. 1978). Shaker (Sh): Sh$^{K121}$ abbreviated as Sh$^{K121}$, Sh$^{K110}$ abbreviated as Sh$^{K110}$, Sh$^{K100}$ with affected transient K$^+$ current $I_A$ (Jan et al. 1977; Salkoff and Wyman 1981; Wu and Ganetzky 1992), Hyperkinetic (Hk): Hk$^{2a}$, Hk$^{K121}$ abbreviated as Hk$^{K121}$ with altered Sh current regulation (Kaplan and Trout 1969; Wang and Wu 1996; Yao and Wu 1999). Shab (Shab$^2$, Shab$^3$) with defects in sustained $I_K$, and $a$ go-go ($eag$): eag$^{2a}$, eag$^{PM}$ defective in multiple K$^+$ currents with major defects in $I_K$ (Ganetzky and Wu 1983; Kaplan and Trout 1969; Wu et al. 1983; Zhong and Wu 1991, 1993). The interactions between bang-sensitive and ion channel mutations were examined in double-mutant combinations with bang senseless (bss$^1$, bss$^2$; Ganetzky and Wu 1982; Jan and Jan 1978): bss$^1$ nap$^{ts}$ (Ganetzky and Wu 1982), bss$^1$ Sh$^{K121}$, and bss$^1$ Sh$^{K110}$ (this study).

**FIG. 1.** Giant fiber (GF) pathway and stimulation protocol for electroconvulsive seizure. A: electrical stimuli were delivered across the brain and physiological responses were recorded at the outputs of the GF pathway. One half of the bilaterally symmetrical GF pathway, which is responsible for jump-and-flight escape reflex, is schematized. GF neuron in the brain activates a jump muscle (TTM) motor neuron (TTMn) directly but recruits flight muscle (DLM) motor neurons (DLMn) by the PSI interneuron in the thorax. Identified synapses (chemical and electrical) are indicated in the pathway. PSI, peripherally-synapsing interneuron; DLMa, dorsal longitudinal muscle a; TTM, tergotrochanteral muscle. B and C: electroconvulsive stimulation protocol. Electroconvulsive stimuli (200 Hz of 0.1-ms pulses) were followed by test pulses (1 Hz of 0.1-ms pulses) to detect failure in nerve conduction and synaptic transmission in the GF pathway during a seizure episode. Stimulus strength was varied by changing voltage (V) or duration (t) of the 200-Hz stimulus train. Examples of seizure responses of a wild-type (B) and a nap$^{ts}$ (C) fly are shown. DLM displayed periods of initial discharge (ID) of seizure spikes, failure (F), and recovery (R) of the GF pathway, and a bout of delayed discharge (DD). In the jump muscle TTM, the same electroconvulsive stimulus also caused a period of response failure, but ID and DD seizure spike activities were not present, indicating that ID and DD seizure discharges do not reflect input from the GF neuron. Note that TTM response recovery is more closely associated with DLM DD onset than with response recovery from GF pathway failure in nap$^{ts}$ flies (C). Closed triangle: onset of delayed discharge DD; open triangle: onset of consecutive evoked responses, indicating complete recovery from GF failure.
Except for Sh, the mutant alleles of different genes examined here displayed similar properties (Supplemental Table 1 and Supplemental Fig. 1) and data from different alleles are pooled to increase sample sizes in Figs. 6–11. Among the Sh alleles, Sh

\(^{133}\) displayed the most striking seizure phenotypes. The results shown in Figs. 6–11 are based on data collected for Sh

\(^{133}\). Some of the mutant strains carried visible markers or balancer chromosomes (single mutants: Sh

\(^{144}/Y\times X\times y\ f\ y\ f\ choose\ cv\ f\ Sh

\(^{20}/Y\times X\times y\ f\ f\ Sh

\(^{44}/Y\times X\times y\ f\ f\ eag\ 4P\ f\ nap^\prime\ cn\ and\ cho\ sn\ bss^2;\ double\ mutants: bss^1\ f/Y\times X\times y^2\ bbs^2; nap^\prime\ cn\ and\ bss^1\ f\ Sh

\(^{155}/Y\times X\times y\ f;\ see\ Lindsley\ and\ Zimm\ 1992\ for\ markers\ and\ balancers).\ Hemizygous\ males\ from\ XX^\prime\ stocks\ were\ examined,\ whereas\ both\ males\ and\ females\ were\ used\ in\ all\ other\ homozygous\ stocks.

Preparation and recording

Tethered flies were used for recordings of electroconvulsive responses as previously described in detail (Lee and Wu 2002). Flies were allowed to recover from ether anesthesia for \(\geq 30\) min and to rest for an additional \(30\) min after electrode insertion before recording. Tungsten electrodes were used for stimulation and recording. A recording electrode was inserted into a dorsal longitudinal muscle (DLM, indirect flight muscle) and sometimes with a second electrode into the tergotrochanteral muscle (TTM, jump muscle) in the thorax, and a reference electrode into the abdomen (Fig. 1A).

The recording electrode penetrated the cuticle into the most dorsal DLM muscle (fiber a) on the right or left side. Although electrodes were uninsulated, only the tips of recording electrodes were inserted into the muscle just beneath the cuticle; thus influence from neighboring muscle fibers was negligible. (Because spike recording was performed with tungsten electrodes, the apparent amplitude of spikes varied with spike frequency and other factors, such as interference from stimulus artifacts.)

Direct triggering of the giant fiber (GF) neuron in the brain evokes short-latency responses in DLM and TTM (Engel and Wu 1992; Tanouye and Wyman 1980). To determine the GF pathway response latency, stimuli (0.1 ms) from a stimulator (Grass SD9 with internal SIU; Grass Instruments, Quincy, MA) were passed by two tungsten electrodes inserted into the eyes. Electroconvulsive stimuli consisted of \(200\) Hz (near the GF following frequency, Engel and Wu 1992), \(0.1\) ms pulse trains (Lee and Wu 2002). After the \(200\)-Hz stimulus, \(1\)-Hz test pulses of \(\geq 24\) V (sufficient to evoke short-latency responses) were applied to examine the response failure and recovery of the GF pathway (Fig. 1, B and C). An interval of \(\geq 10\) min was allowed between applications of high-frequency stimuli, to avoid seizure refractoriness (Lee and Wu 2002). All physiological experiments were performed at room temperature.

Seizure susceptibility was examined for each genotype with four different stimulus-intensity levels (levels 1 to 4 in Figs. 4, 6, 9, and 11): \(50\) V, \(0.5\) s; \(50\) V, \(1.0\) s; \(50\) V, \(2.0\) s; \(100\) V, \(2.0\) s (representing a progressive doubling of intensity). For bang-sensitive mutants with low seizure thresholds, lower intensity levels were also included: \(50\) V, \(0.4\) s; \(50\) V, \(0.2\) s; \(50\) V, \(0.1\) s (representing a progressive halving). We previously documented that doubling the stimulation train duration has an effect similar to doubling the stimulus voltage in the same fly (Lee and Wu 2002). Using fixed intensity steps at a compressed log scale (base 2) allowed the coverage of a broader stimulus intensity range and facilitated a standardized characterization of the seizure repertoire among a large number of flies. Thus different seizure

\(^{1}\) The online version of this article contains supplemental data.
repertoire components (ID, DD, and F) could be compared at each defined intensity level across genotypes. Variable stimulus voltages were used in Figs. 2 and 5 to illustrate the voltage dependency of the seizure repertoire at a linear scale.

Threshold levels for seizure induction were estimated from the read-out of 1/2 probability levels (i.e., an estimate of the median) from Figs. 4, 6, 9, and 11 or from the mean of the minimum intensity level required to evoke DD in individual flies (Supplemental Fig. 1). Both methods led to similar conclusions. Computer-assisted statistical computations of SD, SE, t-test, and linear regression were performed with the software Excel.

**Spike pattern analysis**

DLM spike responses were picked up with an AC preamplifier (filter bandwidth from 0.1 Hz to 30 kHz, reference grounded; DAM-5A; World Precision Instruments, New Haven, CT) and recorded with pulse code modulation (DR-384; NeuroData, New York, NY) on videotapes at a sampling rate of 44 kHz. Gap-free recording mode of the Fetchex program in pClamp6 software (Axon Instruments, Foster City, CA) was used for digital data acquisition (1 kHz) on a Pentium computer equipped with Digidata 1200 (Axon Instruments). Spikes were detected using Axograph 3.0 software (Axon Instruments).

**RESULTS**

**Signature components of the electroconvulsive seizure repertoire and effects of reduced Na⁺ channel expression**

As previously reported (Lee and Wu 2002), electroconvulsive stimulation (200 Hz, followed by 1-Hz test pulses) applied across the brain induces a stereotypic seizure repertoire with a succession of distinct components observed in the flight muscle DLMs: initial discharge (ID), response failure (F, to 1-Hz test pulses), and delayed discharge (DD) of seizure spikes (Fig. 1B). DLM motor neurons receive inputs from the GF neuron through a peripherally synapsing interneuron (PSI) and can be driven by inputs from additional circuits. Electroconvulsive stimulation does not evoke high-frequency discharge in the jump muscle (TTM), whose motor neuron is directly innervated by the GF (Fig. 1A). Thus the sources of ID and DD spikes in DLM recordings are independent of the GF neuron but are derived from additional resonant circuits, presumably involving the flight pattern generator (Lee and Wu 2002). However, recovery of TTM response failure does not necessarily coincide with recovery of DLM response failure but is more closely associated with the onset of DD seizure spikes in DLMs in a variety of genotypes (Lee and Wu 2002), including...
nap\textsuperscript{ts} (Fig. 1, B and C). Therefore DD onset signifies recovery of several robust neural elements including the GF neuron and some resonant circuits.

Figure 2 illustrates the progressive expression of the electroconvulsive seizure repertoire at increasing stimulus intensities in a wild-type fly and a hypoexcitable nap\textsuperscript{ts} mutant with reduced Na\textsuperscript{+} channel density (Wu and Ganetzky 1992). We observed the effects of near- and suprathreshold electroconvulsive stimuli using a sequence of 200-Hz stimulus trains, delivered in 10-V increments, each followed by a train of 1-Hz test pulses to monitor the failure and recovery of the giant fiber (GF) pathway. In the absence of brain stimulation, tethered flies of different genotypes display sporadic, spontaneous, high-frequency spikes associated with nonflight, cleaning behaviors (Fig. 2, top traces; cf. Banerjee et al. 2004). Spontaneous discharges (brackets above traces) became totally suppressed at suprathreshold levels that evoked full-blown DD expression (Fig. 2). In contrast to ID and DD that have been shown to reflect global CNS activity by simultaneous recordings (Lee and Wu 2002), these spontaneous discharges were not associated with general seizure activity.

In flies of a variety of genotypes, ID displays a nonlinear stimulus–response relation (Fig. 2A; cf. Lee and Wu 2002). Above a threshold level, ID was progressively lengthened by increments of stimulus intensity but became shortened and even completely suppressed on further increases in stimulus intensity. In contrast, the durations of DD seizure exhibited a monotonic increase with stimulus intensities, reaching a plateau response at an intensity beyond that of maximum ID expression (60 V in Fig. 2A), defining the maximum expression level of the DD central pattern generator as well as the minimum time required for GF pathway and TTM recovery from failure (cf. Fig. 1).

In nap\textsuperscript{ts} flies, higher stimulus intensity was required to induce an electroconvulsive response (Fig. 2B). Most significantly, the nap\textsuperscript{ts} mutation completely eliminated the first bout of seizure discharge (ID), shortened the duration of second bout of discharge (DD), and drastically lengthened the period of GF pathway failure (F). Nevertheless, as in wild-type flies, DD onset, F, and DD duration in nap\textsuperscript{ts} clearly displayed plateau expression beyond certain stimulus intensity (90 V in Fig. 2B). The results indicate that ID, DD, and F represent three independent signature components of the electroconvulsive seizure repertoire, whose induction and expression are modifiable by genetic and physiological manipulations.

The spontaneous discharges and ID and DD firing patterns are distinguishable by their frequency characteristics as indicated in the instantaneous spike frequency plots (Fig. 3A, based on data shown in Fig. 2). In general, the peak frequency of ID (about 60 Hz) was greater than that of DD (about 20 Hz), whereas the spontaneous discharges were highly irregular and could reach a peak frequency >100 Hz. In nap\textsuperscript{ts} flies, the spontaneous discharges were not apparently affected even though DD was severely suppressed and ID was eliminated (Fig. 3B; cf. Fig. 8 below).

We constructed probability plots for ID, GF pathway failure, and DD at defined stimulus-intensity levels (see METHODS) based on the total samples of WT and nap\textsuperscript{ts} flies (Fig. 4). Such probability plots facilitated quantitative comparisons among ID and DD induction and GF pathway failure across different genotypes (cf. Figs. 6 and 9). It is evident that nap\textsuperscript{ts} flies showed decreased susceptibility to electroconvulsion, as indicated by greatly reduced probability of DD induction and GF pathway failure, plus the absence of ID, at the different stimulus levels examined (Fig. 4). This result is consistent with the previous report of extremely high threshold voltage for ID induction in nap\textsuperscript{ts} flies (Kuebler et al. 2001). This abnormally high seizure threshold is correlated with drastic changes in the...
expression of seizure components, DD and GF pathway failure (Figs. 2 and 3).

Differential mutational effects of K⁺ channel subtypes on the components of electroconvulsive seizure

We subsequently examined the electroconvulsive seizure repertoire in hyperexcitable K⁺ channel mutants. Several alleles of the Sh, Hk, Shab, and eag genes were studied (see METHODS). Representative near-threshold and plateau-level responses are shown for Sh<sup>133</sup>, Hk<sup>1</sup>, Shab<sup>9g</sup>, and eag<sup>1</sup> flies in Fig. 5.

At near-threshold stimulus levels, ID was first initiated, along with sporadic spontaneous discharges (Fig. 5A, brackets), but GF pathway failure and DD induction rarely occurred. Again, at sufficiently high stimulus intensities (Fig. 5B, suprathreshold), ID was suppressed, whereas DD expression and GF pathway failure (F) reached plateau levels in all genotypes. Even so, these signature seizure components were differentially modified by each K⁺ channel mutation (Fig. 5B; cf. Fig. 7 below).

Of the mutations that affect voltage-activated K⁺ currents, Sh and Hk alter the α- and β-subunits, respectively, of the Shaker channel that mediates transient I<sub>K</sub>. Shab and eag mutations affect different K⁺ channels that regulate components of sustained I<sub>K</sub>. Interestingly, Sh and Hk flies displayed continuous, vigorous leg shaking under ether anesthesia, whereas only mild leg shaking and other uncoordinated behaviors are seen in Shab and eag mutants under the same condition.

We quantified the stimulus intensity dependency of seizure induction in the various K⁺ channel mutants. The probability of induction of the signature components—ID, DD, and GF pathway failure—was determined at different stimulus-intensity levels (Fig. 6; see METHODS). Except for Sh<sup>133</sup> flies from multiple alleles of each genotype were pooled to construct the intensity–response curves (Fig. 6) and the extent of expression charts (Fig. 7 below). Unlike other K⁺ channel mutations, Sh mutations displayed a clear allele-dependent effect. Among the Sh alleles examined, Sh<sup>133</sup> displayed the most extreme expression of seizure components and data from this allele are presented in these figures (see METHODS and compare allele differences in Supplemental Table 1 and Supplemental Fig. 1).

Interestingly, Sh<sup>133</sup> and Hk mutations preferentially enhanced ID induction, whereas Shab and eag mutations retarded induction of DD seizure and GF pathway failure, resembling the nap<sup>α</sup> effects but to a lesser extent (compare Fig. 4). Unlike the drastic effects of nap<sup>α</sup> on all signature components, Sh<sup>133</sup> and Hk did not obviously alter the thresholds for DD seizure and GF pathway failure induction and the effects of Shab and eag on ID induction were relatively mild (Fig. 6). The mutational effects for seizure susceptibility were consistent when we compared minimum stimulus intensity for DD induction (Supplemental Fig. 1).

Comparisons of Na⁺ and K⁺ channel mutations in their modifications of electroconvulsive seizure expression

In addition to seizure susceptibility, we quantified extents of modifications in the expression of the seizure repertoire by different ion channel mutations. Expressions of ID in a variety of bang-sensitive and K⁺ channel mutants have been well documented (Kuebler et al. 2001; Pavlidis and Tanouye 1995). We focused on the maximum expression levels of other signature events of the electroconvulsive repertoire, that is, GF pathway failure duration, time to DD onset, and DD seizure duration (Fig. 7). We took advantage of the relatively consis-
tent expression of their plateau responses so that there was no need to precisely control the stimulus level to obtain highly reproducible measurements within individual genotypes.

Hypoexcitable nap\\textsuperscript{t} mutants with the reduced seizure susceptibility showed significantly reduced DD seizure duration and, once successfully evoked, recovery from GF pathway failure was delayed. In contrast, distinct categories of hyperexcitable K\textsuperscript{+} channel mutations exerted differential effects on DD onset time and the durations of DD seizure and GF pathway failure. Apparently, defects in sustained I\textsubscript{K} in Shab and eag mutants had more profound effects on the seizure repertoire than defective transient I\textsubscript{A} in Sh and Hk mutants (Fig. 7). Unlike nap\\textsuperscript{t}, K\textsuperscript{+} channel mutations, in various degrees, postponed DD onset time and lengthened the durations of both DD seizure and GF pathway failure. As mentioned above (Fig. 1), DD onset time signifies roughly the time of recovery from electroconvulsion-induced failure of the GF neuron and other more robust neural elements such as the central pattern generator of DD (Lee and Wu 2002). In contrast, recovery from GF pathway failure, lagging behind DD onset in different mutants, involves recovery of the downstream elements, the PSI and the DLM motor neuron. Significantly, the most striking lengthening of all three signature events was observed in Shab mutants, whereas no modifications were detected in Hk mutants (Fig. 7). However, different K\textsuperscript{+} channel mutations, including Hk, exerted significant influences on DD spike patterns (Fig. 8).

We investigated how ion channel mutations modify the central pattern generator of DD spike patterns by analyzing instantaneous spike frequency (Fig. 8A) and other temporal characteristics (Fig. 8B). As expected, nap\\textsuperscript{t} severely reduced in the peak frequency and total number of spikes generated during DD seizure. Unexpectedly, Hk followed the trend of nap\\textsuperscript{t} in suppressing DD seizure, whereas Sh, Shab, and eag showed opposite effects in increasing the total number of spikes and the time to peak expression of DD seizure without altering peak DD frequency.

**Interactions between ion channel and bang-sensitive mutations**

Another approach to establishing the roles of membrane excitability in seizure susceptibility and expression is to investigate the effects of channel mutations on the phenotype of bang-sensitive mutants that are known to exhibit the most striking repertoire under various stress conditions. Representative examples present the phenotypic modifications in the double mutants bss\textsuperscript{1} nap\\textsuperscript{t} and bss\textsuperscript{1} Sh\textsuperscript{t33}. As previously described (Lee and Wu 2002), bss mutants show extreme sensitivity to electroconvulsive stimuli, with the hallmarks of

![Stimulus intensity–response relationships of ID and DD seizure induction and GF pathway failure in different K\textsuperscript{+} channel mutants. I\textsubscript{A} channel mutants Sh and Hk showed increased probability in ID induction at different stimulus intensity levels. In contrast, GF pathway failure (GF pathway F) and DD induction probability were suppressed by mutations of I\textsubscript{K} channels, Shab and eag. Sample sizes for each genotype are indicated. See Fig. 4 for stimulus intensity determination.](http://jn.physiology.org/doi/10.1063/1.2220336)
greatly increased DD duration, time to DD onset, and GF pathway recovery time (cf. Fig. 10 below). Decreased Na\textsuperscript{+} channel numbers caused by the nap\textsuperscript{G} mutation suppressed seizure expression (Fig. 9A) and susceptibility to electroconvulsion (Fig. 9B). In bss nap\textsuperscript{G} flies, stimulus-intensity dependency curves of GF pathway failure and DD seizure induction were partially restored toward the wild-type levels (Fig. 9B).

Similarly, combining nap\textsuperscript{G} with other bang-sensitive mutations in bss nap\textsuperscript{G}, bas nap\textsuperscript{G}, eas nap\textsuperscript{G}, and tko nap\textsuperscript{G} double mutants produced this trend (data not shown). In fact, this is consistent with the behavioral observation that seizure in bang-sensitive flies caused by mechanical disturbances was suppressed by nap\textsuperscript{G} as shown in bss nap\textsuperscript{G}, bas nap\textsuperscript{G}, eas nap\textsuperscript{G}, and tko nap\textsuperscript{G} double mutants (Ganetzky and Wu 1982). In contrast, Sh\textsuperscript{1/33} mutations exerted opposing effects of nap\textsuperscript{G} by further enhancing seizure susceptibility in bss Sh\textsuperscript{1/33} double-mutant flies (Fig. 9B and Supplemental Fig. 1). Notably, Sh\textsuperscript{M} also enhanced seizure susceptibility in bss Sh\textsuperscript{M}, even though unlike Sh\textsuperscript{1/33}, Sh\textsuperscript{M} showed a threshold higher than that of wild-type (Supplemental Fig. 1).

These results support the notion that bang-sensitive mutations are associated with increased excitability, as demonstrated in the larval neuromuscular preparation that repetitive stimuli induce abnormal multiple firing in bss\textsuperscript{1}, which is suppressed by nap\textsuperscript{G} in bss\textsuperscript{1} nap\textsuperscript{G} (Ganetzky and Wu 1982). However, the properties of bss\textsuperscript{1} nap\textsuperscript{G} and bss\textsuperscript{1} Sh\textsuperscript{1/33} double mutants highlighted the nonlinear interactions between ion channel and bang-sensitive mutations. Decreased excitability of nap\textsuperscript{G} significantly suppressed reduced DD seizure duration in bss\textsuperscript{1} nap\textsuperscript{G} but further enhancement of excitability by Sh actually decreased DD duration as well in bss\textsuperscript{1} Sh\textsuperscript{1/33} and bss\textsuperscript{1} Sh\textsuperscript{M} (Fig. 10A and Supplemental Table 1). Moreover, the recovery time from GF pathway failure was significantly decreased in bss\textsuperscript{1} nap\textsuperscript{G}, even though both nap\textsuperscript{G} and bss mutations lengthened the GF pathway recovery time (Fig. 10B).

A hallmark of stress-induced seizure in bang-sensitive mutants (Ganetzky and Wu 1982) and electroconvulsion-induced seizure in a variety of genotypes (Lee and Wu 2002) is the refractory period that reflects the gradual process of regaining seizure susceptibility subsequent to a seizure episode. At different time points during the refractory period, the degree of responsiveness to electroconvulsion serves as an indicator for the rate of regaining seizure susceptibility in different genotypes. Thus paired electroconvulsive stimuli 1 min apart were used to determine the effects of nap\textsuperscript{G} and Sh on the bss refractory period. A unique trait of bss flies is an extremely short refractory period, as indicated by a nearly 80% recovery within the 1-min rest period. Significantly, neither nap\textsuperscript{G} nor Sh modified the rapid recovery of bss in bss\textsuperscript{1} nap\textsuperscript{G} and bss\textsuperscript{1} Sh\textsuperscript{1/33}, although both nap\textsuperscript{G} and Sh showed a wild-type speed of regaining seizure susceptibility (Fig. 10C). Therefore the phenomenon of refractoriness is relatively insensitive to modification by excitability levels, unlike the strong influences of both nap\textsuperscript{G} and Sh mutations on DD seizure and GF pathway failure expression (Fig. 10, A and B).

**Correlations among components of the seizure repertoire**

To gain further insights into the induction and expression of the seizure repertoire, we sought coupling and independence of the effects on the individual components by the various mutations described above. We examined correlations among all possible pairs of the parameters based on the data shown in Figs. 4, 6, 7, 9B, and 10. The tightest coupling was found between stimulus thresholds for DD seizure induction and GF

![Fig. 7. Summary of differential modifications of the electroconvulsive seizure repertoire in Na\textsuperscript{+} and K\textsuperscript{+} channel mutants. Recovery time from GF pathway failure (F duration) and the onset time and duration of delayed discharge (DD) were quantified for plateau expression in each genotype. Each parameter (F duration, DD onset, and DD duration) could be differentially modified by the various mutations, suggesting different neural elements or circuits responsible for the individual seizure components. F and DD durations are more prone to mutational modifications, showing enhancement or suppression in different mutants. Note that only increases in GF pathway failure and DD onset time were observed in the various mutations, indicating that the wild-type parameters represent a ground state of optimal conditions for recovery from electroconvulsion. Sample size shown in the bar indicates the number of flies, each contributing to a single repertoire, used for computing the mean and SD for the parameters in each genotype. *P < 0.05 against WT; **P < 0.01; ***P < 0.001.](http://jn.physiology.org/10.1152/jn.00515.2006)
pathway failure. The threshold levels for these seizure repertoire components were estimated from the read-outs of 1/2 probability levels from Figs. 4, 6, and 9 (see METHODS). The correlation between stimulus levels for 1/2 probability of DD seizure induction and 1/2 probability of GF pathway failure was as high as \( r^2 / H11005 0.99 \) among all genotypes examined, including bss and the double mutants (Fig. 11A).

We also examined seizure susceptibility based on the minimum stimulus intensity levels required to evoke DD seizure among the genotypes (Supplemental Fig. 1A). This method led to the same sequence of susceptibility as shown in Fig. 11A, with Shab and nap\(^{33}\) being the most resistant and bss and bss Sh the most sensitive to electroconvulsion (compare Fig. 11A and Supplemental Fig. 1B). The same is true for the minimum intensity levels for the induction of GF pathway failure (data not shown), confirming the tight coupling between the induction of DD seizure and GF pathway failure. The strong correlation illustrates the simple fact that the occurrence of the seizure repertoire is all or none, characterized by the fixed sequence of the signature events.

A weaker correlation could be observed between DD onset time and duration required for recovery from GF pathway failure (Fig. 11B). Note that DD onset signifies the beginning of activity recovery in the GF neuron, jump muscle, and central pattern generator for DD, whereas duration of GF pathway failure also includes recovery of the GF neuron plus PSI and DLM motor neuron. Therefore the mutational effects on these two events are expected to show a certain correlation (Fig. 11B, \( r^2 = 0.77 \) among all genotypes).

However, very poor or no correlation was observed between other pairs of seizure components. For example, duration of GF pathway failure (inactivity period) and DD seizure duration (reflecting the central pattern generator activity) yielded a correlation of \( r^2 = 0.17 \) (Fig. 11C). Similarly, the remaining pairs—DD duration versus GF pathway failure threshold, DD onset time versus GF pathway failure threshold, and F duration versus GF pathway failure threshold—all yielded insignificant \( r^2 \) values. This represents an interesting parallel with a previous study (Kuebler et al. 2001), which also demonstrated that the threshold and following frequency of the GF-mediated DLM response, two basic excitability properties of the GF, show no simple correlations with the threshold for ID induction among a number of ion channel and bang-sensitive mutations.

**DISCUSSION**

This study provides direct evidence that the expression of both electroconvulsion-induced and classical bang-sensitive seizure can be modified by mutations affecting Na\(^+\) and K\(^+\) currents in *Drosophila*. The results demonstrate that individual ion channel subtypes play separate roles in determining seizure susceptibility (cf. Kuebler et al. 2001) and shaping seizure discharge patterns that originate from specific neural circuits (cf. Lee and Wu 2002). Thus the alterations by Na\(^+\) and K\(^+\) channel mutations described here can provide a basis for further experiments to gain a better understanding of seizure mechanisms.

**Na\(^+\) channel mutations**

The nap\(^{33}\) mutation decreases the expression of the para gene that encodes a Na\(^+\) channel subtype (Kernan et al. 1991).
reducing Na\(^+\) channel density to about 50% as determined by toxin binding (Jackson et al. 1984; Kauvar 1982). Compared with K\(^+\) channel mutations, the napts effects were especially striking and pervasive, including increased seizure threshold, suppressed DD expression, complete inhibition of ID seizure, and delayed GF pathway recovery (Figs. 2, 4, 7, and 8). The paralytic phenotype of the napts mutation is apparent only at high temperature (Wu et al. 1978). However, at room temperature the hypoexcitability of the napts mutation is indicated by its double-mutant suppression of the leg-shaking behavior in Sh and Hk mutants as well as the stress-induced seizure in bang-sensitive mutants (Ganetzky and Wu 1982). The modified electroconvulsion-induced seizure therefore represents a sensitive indicator for the physiological phenotype at room temperature in napts single mutants.

The napts effects on electroconvulsive seizure can be attributed to modified neuronal, rather than muscle, membrane excitability. In Drosophila and other invertebrate species, neurons use Na\(^+\) channels for action potential generation but muscle excitation depends on Ca\(^{2+}\) rather than on Na\(^+\) channels (Singh and Wu 1999). It was previously shown that mutant alleles of para with altered Na\(^+\) channel functions (as opposed to reduced density of normal Na\(^+\) channels described here) also decreased susceptibility to electroconvulsion (Kuebler et al. 2001). Another Na\(^+\) channel structural gene, DSC (Salkoff et al. 1987), also exists in Drosophila and exhibits expression patterns different from those of para (Hong and Ganetzky 1994). The activation and inactivation properties of these two Na\(^+\) channel types may differ and it will be of interest to determine whether they exert distinct effects on individual components of seizure repertoire that originate from separate anatomical loci (Lee and Wu 2002).

Our results imply that excessive Na\(^+\) channel density or enhanced Na\(^+\) channel activity, such as by slowed inactivation, can make animals seizure prone. Certain neuronal elements in the nervous system may be endowed with a high density of Na\(^+\) channels. For example, the squid giant axons express an excessively high density of Na\(^+\) channels for a safety margin in preventing conduction failure (Hille 1992). Apparently, the GF neuron, a major element in the escape reflex circuit of Drosophila, may also express a high density of para and DSC Na\(^+\) channels. Notably, the napts mutation lengthened the GF pathway failure duration but did not alter the DD onset time (Fig. 7). DD onset indicates recovery of GF neuron activity suppressed by electroconvulsion, whereas GF pathway failure duration involves recovery of additional downstream circuit elements, i.e., the PSI and DLM motor neurons (Fig. 1A).

**K\(^+\)** channel mutations

Our study indicates that individual components of the seizure repertoire are differentially affected by mutations of...
nevertheless, the expression of seizure repertoire was enhanced by these K⁺ channel mutations to various extents, including increased durations of GF pathway failure and DD seizure as well as the time to DD onset (Fig. 7). Notably, Shab mutations exerted the most extreme effects.

Compared with nap⁺, the effects of K⁺ channel mutations on the components of seizure repertoire were relatively mild. K⁺ channels are more diverse than Na⁺ channels in molecular subtypes with different kinetic properties and voltage dependency (Coetzee et al. 1999). It is possible that functional disruptions by elimination of a particular K⁺ channel subtype may be mitigated by other K⁺ channel subtypes with partially overlapping properties. For example, null mutations of para Na⁺ channels cause lethality but null alleles of Sh and Shab are viable and relatively healthy. In Drosophila, additional components of transient Iₐ and sustained Iₖ are mediated by the K⁺ channel subtypes encoded by Shal and Shaw, respectively. It will be important to determine functional divisions among these K⁺ channel subtypes in the neural circuits responsible for seizure generation.

Expression patterns of individual channel subunits can contribute to the diverse mutant phenotypes. Although Sh and Hk separately code for the α- and β-subunits of the Sh Iₐ channels, their mutational effects are not identical, e.g., Hk produced far fewer DD spikes than that of Sh (Fig. 8). In cultured neurons, Hk mutations cause hyperexcitability only in a subpopulation and individual mutant neurons can display distinct categories of spike patterns, some of which are not observed in Sh cultures (Yao and Wu 1999). Therefore it will be important to determine the exact expression patterns of Hk and Sh in the circuits responsible for each component of the seizure repertoire in future studies.

Another potential source of diverse mutational effects of K⁺ channels is the presence of RNA splice mechanisms for some K⁺ channel genes. It was previously documented that Sh RNA splicing produce a number of distinct Sh channel subunits (Iverson et al. 1988; Kamb et al. 1987; Papazian et al. 1987; Schwarz et al. 1988). A considerable range of severity has been shown in Sh alleles for action potential duration in the GF neuron (Tanouye and Ferrus 1985) and for DLM response to GF neuron activation (Engel and Wu 1992), a variation that may be related to differential effects on distinct splice variants. Unlike other K⁺ channel genes, Sh displayed clear allele-dependent mutational effects.

Among the different Sh alleles examined, Sh¹³³ stood out with the most striking expression of altered DD and F. Interestingly, Sh¹³³ also showed the lowest threshold for evoking a seizure repertoire. In contrast, the data of Sh⁵ and Sh⁴ (but not Sh¹³³) indicate an increased threshold level of DD induction (Supplemental Fig. 1), even though these mutations do not appear to alter seizure repertoire expression (Supplemental Table 1).

An independent study by Kuebler and associates (2001) reported increased thresholds of ID induction in nap⁺ and para⁺ as well as eag, in general agreement with our observations on modifications of ID, DD, and F. Interestingly, three Sh alleles (Sh¹³³, Sh¹, and Sh¹²⁰) are reported to increase ID threshold and Sh¹³³ is found to suppress sda, a bang-sensitive mutation, in Sh sda double mutants (Kuebler et al. 2001). This contrasts with our finding of increased seizure susceptibility in Sh¹³³ and its enhancing effects on bss seizure sensitivity in bss

**FIG. 10.** Modifications of parameters of the bss seizure repertoire by nap⁺ and Sh. A: prolonged duration of DD seizure spike activity caused by the bss' mutation was suppressed by nap⁺ in bss¹ nap⁺, but also significantly decreased by Sh¹³³ in bss¹ Sh¹³³. B: duration of extended GF pathway failure caused by bss' was restored to near normal in bss¹ nap⁺ but remained extremely prolonged in bss¹ Sh¹³³. C: invariant refractory period in bss¹ single and double mutants. During the relative refractory period after an electroconvulsive seizure, paired suprathreshold stimuli (2-s trains of 200 Hz, 0.1 ms at 50 V) were delivered 1 min apart to monitor the extent of GF pathway recovery. Normalization of the second F duration to the first reflects the degree of recovery from refractoriness. Recovery at 1 min in Sh¹³³ and nap⁺ mutant flies did not differ from that of wild-type flies. Recovery was strikingly fast in bss¹ mutants, which regained nearly full responsiveness and remarkably this rapid recovery was not modified by nap⁺ and Sh¹³³ in double-mutant combinations. *P < 0.05; ***P < 0.01; ****P < 0.001, against WT; #P < 0.01; ##P < 0.001, against bss.

5 different K⁺ channel subtypes. Sh¹³³ and Hk, affecting transient Iₐ, promoted induction of ID seizure but Shab and eag, affecting sustained Iₖ, increased threshold for GF pathway failure and DD seizure induction (Fig. 6). Once triggered,
J. LEE AND C.-F. WU

FIG. 11. Correlations among electroconvulsive seizure parameters. A: strong positive correlation between $F_{1/2}$ and $DD_{1/2}$ stimulus levels ($r^2 = 0.99$, slope = 1.0). Stimulus intensities required for F and DD induction in 50% of flies are derived from data presented in Figs. 4, 6, and 9B. Tight coupling between the 2 events reflects the all-or-none nature of the seizure repertoire. B: weaker positive correlation between time to DD onset and GF pathway failure duration ($r^2 = 0.77$). DD onset signifies regaining of DD central pattern generator activity and F generation indicates recovery from transmission failure involving GF neuron, PSI and DLM motor neuron. C: lack of correlation between DD seizure and GF pathway failure durations ($r^2 = 0.17$). Similarly, no significant correlations were observed between other pairs of combinations among the electroconvulsive seizure parameters shown in Figs. 7 and 10. Linear regression lines are shown for visual aid. Genotypes are indicated next to the data points.

Sh double mutants (Figs. 9 and 10). The reason for this discrepancy cannot be certain at the present time. The possibilities include potential differences in the genetic background and the sources of the mutant stocks, as well as the different electroconvulsion protocols (0.5- vs. 0.1-ms pulse duration in the 200-Hz stimulus train) and indicators (ID vs. DD) used for seizure measurements in the two studies. Nevertheless, we found both $bss Sh^{M}$ and $bss Sh^{133}$ double mutants showed a threshold lower than that of $bss$, despite the different seizure threshold levels of $Sh^{M}$ and $Sh^{133}$ (Supplemental Fig. 1), contrary to the case of $Sh sda$ interaction. Moreover, $sda nap^{a}$ shows a high-threshold level, close to that of $nap^{a}$, whereas $bss nap^{a}$ threshold is restored toward the wild-type level (compare Kuebler et al. 2001 and Fig. 9 and Supplemental Fig. 1). Our conclusions are also consistent with the more extreme bang-sensitive behavior of $bss Sh^{133}$ and $bss Sh^{M}$ as well as the further enhanced multiple firing phenotype of $bss$ (Ganetzky and Wu 1982) at $bss Sh^{133}$ larval neuromuscular junctions (data not shown).

Another consideration is that interactions between K\(^+\) channel mutations and bang-sensitive mutations depend on the nature of particular bang-sensitive genes, which have a broad heterogeneity. The contrasting effects of $Sh$ on $sda$ and $bss$ in $Sh sda$ and $bss Sh$ double mutants is paralleled by $bss$ and $sda$ interactions with the gap junction mutation Shaking $B$ (Shak $B$). The seizure threshold in Shak$B sda$ is between that of the corresponding single mutants, whereas the threshold for $bss ShakB$ is bss-like (Song et al. 2006). The $sda$ gene codes for an aminopeptidase (Zhang et al. 2002) but the molecular lesion of $bss$ is still unknown.

Characteristics of the seizure repertoire

The signature components in the electroconvulsive seizure repertoire are separable by surgical manipulation and gynandromorph mosaic dissection (Lee and Wu 2002), consistent with their differential modifications by Na\(^+\) and K\(^+\) channel mutations described here. The seizure repertoire is evoked in an all-or-none manner, as reflected by the tight coupling between the induction probability of DD seizure and GF pathway failure among the various genotypes (Fig. 11A). However, few correlations could be found between the extents of modification in DD seizure and GF pathway failure among genotypes. A significant correlation was found only between the time to DD seizure onset and recovery from GF pathway failure (Fig. 11B). These two events signify the recovery of activities of the various circuit components from depression caused by electroconvulsion. DD seizure onset correlates with the GF neuron recovery and a “rebound” of spike activity from a central pattern generator (Fig. 1; cf. Lee and Wu 2002). One remarkable observation related to these events is that only increases, but no decrease, of these quiescent periods were caused by Na\(^+\) or K\(^+\) channel mutations. That is, the wild-type nervous system appears to be optimal in recovery from electroconvulsive shock, as though the wild-type values represent those of a ground state that is maintained by a delicate balance between excitation and repolarization forces.

It is apparently counterintuitive that some K\(^+\) channel mutations should increase GF pathway failure duration as the $nap^{a}$ mutation does. However, it has been shown in the GF pathway that hyperexcitability in K\(^+\) channel mutations ($Sh^{120}$ and $Sh^{145}$) and double mutations ($eag^{a} Sh^{120}$ and $eag^{a} Sh^{133}$) cause unexpected defects in DLM and TTM responses, such as increased action potential refractory period and reduced following frequency that are also observed by $nap^{a}$ (Engel and Wu 1992). Similar changes in following frequency of DLM...
have also been reported for \(Sh^5\), \(Sh^{120}\), and \(Sh^{133}\) (Kuebler et al. 2001). Presumably, lack of repolarization forces in certain \(K^+\) channel mutants can lengthen the recovery time of \(Na^+\) channel inactivation, resulting in an effect similar to that caused by reduced \(Na^+\) currents in nap\(^{17}\).

Different categories of molecular lesions have been implicated in epileptic seizure, including ligand-gated receptor channels in addition to voltage-gated ion channels (Scheffer and Berkovic 2003; Steinlein and Noebels 2000). Classical bang-sensitive mutants in \textit{Drosophila} indicate a potentially greater heterogeneity in the molecular mechanisms that may be involved in modifications of seizure susceptibility and expression. The products of bang-sensitive genes include a heterogeneity of molecular categories, such as ethanolamine kinase of \textit{eas} (Pavlidis et al. 1994), mitochondrial ribosomal protein of \(tko\) (Royden et al. 1987), and aminopeptidase of \(sda\) (Zhang et al. 2002). These mutations greatly enhance seizure susceptibility as demonstrated by lowered threshold for electroconvulsive seizure (Fig. 9; see Kuebler et al. 2001; Lee and Wu 2002). Furthermore, in classical bang-sensitive mutants, seizure is triggered by mechanical disturbance and other stress conditions, a phenotype that is not phenocopied by any of the mutations examined here. Nevertheless, our results demonstrate that the expression of the susceptibility and seizure expression of bang-sensitive mutants can be modified by \(Na^+\) and \(K^+\) channel mutations. This highlights the importance of understanding the complex molecular interactions in seizure generation within individual neuronal populations and specific neural circuits responsible for the separate components of the seizure repertoire.

Future investigation should elucidate anatomical loci, the identity of neuronal elements, and central pattern generators underlying the seizure repertoire. A battery of genetic and molecular tools is now available in \textit{Drosophila} for targeted or conditional expression of transgenes. Either normal or mutant copies of channel transgenes can be expressed using the Gal4-UAS system in particular neuronal populations (Brand and Perrimon 1993). Mutations causing epileptic seizure in humans and vertebrate model systems often implicate altered excitability caused by mutations of several \(Na^+\) and \(K^+\) channel subtypes (Scheffer and Berkovic 2003; Steinlein and Noebels 2000). Therefore the information gained from studies on \textit{Drosophila} channel mutants may facilitate development of therapeutic strategies for certain categories of epileptic seizure.

**Acknowledgments**

We thank Dr. Jeff Engel for generous help during this study and Dr. Satpal Singh for providing \textit{Shab} mutant alleles.

**References**


