Drosophila as a Model for Intractable Epilepsy: Gilgamesh Suppresses Seizures in \( \text{para}^{bss1} \) Heterozygote Flies

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ABSTRACT

Intractable epilepsies, that is, seizure disorders that do not respond to currently available therapies, are difficult, often tragic, neurological disorders. Na⁺ channelopathies have been implicated in some intractable epilepsies, including Dravet syndrome (Dravet 1978), but little progress has been forthcoming in therapeutics. Here we examine a Drosophila model for intractable epilepsy, the Na⁺ channel gain-of-function mutant \( \text{para}^{bss1} \) that resembles Dravet syndrome in some aspects (Parker et al. 2011a). In particular, we identify second-site mutations that interact with \( \text{para}^{bss1} \), seizure enhancers, and seizure suppressors. We describe one seizure-enhancer mutation named charlatan (chn). The chn gene normally encodes an Neuron-Restrictive Silencer Factor/RE1-Silencing Transcription factor transcriptional repressor of neuronal-specific genes. We identify a second-site seizure-suppressor mutation, \( \text{gilgamesh} \) (gish), that reduces the severity of several seizure-like phenotypes of \( \text{para}^{bss1/+} \) heterozygotes. The gish gene normally encodes the Drosophila ortholog of casein kinase CK1g3, a member of the CK1 family of serine-threonine kinases. We suggest that CK1g3 is an unexpected but promising new target for seizure therapeutics.

KEYWORDS

sodium channel epilepsy seizure-suppression Drosophila

In this study, we examine genetic complexities that underlie seizure-susceptibility by using, as a model, genetic combinations of single-gene mutations in the fruit fly Drosophila: seizure-sensitive, seizure-enhancer, and seizure-suppressor mutations. The study is based on genetic interactions that modify phenotypes in \( \text{para}^{bss1} \), a model for intractable epilepsy (Parker et al. 2011a). The \( \text{para}^{bss1} \) allele is a gain-of-function mutation caused by a substitution (L1699F) of a highly conserved residue in the third membrane-spanning segment (S3b) of homology domain IV (Parker et al. 2011a). In this study, we examine genetic complexities that underlie seizure-susceptibility by using, as a model, genetic combinations of single-gene mutations in the fruit fly Drosophila: seizure-sensitive, seizure-enhancer, and seizure-suppressor mutations. The study is based on genetic interactions that modify phenotypes in \( \text{para}^{bss1} \), a model for intractable epilepsy (Parker et al. 2011a). The \( \text{para}^{bss1} \) allele is a gain-of-function mutation caused by a substitution (L1699F) of a highly conserved residue in the third membrane-spanning segment (S3b) of homology domain IV (Parker et al. 2011a).

MATERIALS AND METHODS

Fly stocks

Drosophila strains were raised on standard cornmeal-molasses agar medium at room temperature (23–25°C). The \( \text{para} \) gene is located at map position 1–53,5 and encodes a voltage-gated Na⁺ channel (Loughney et al. 1989; Ramaswami and Tanouye, 1989). The bang-sensitive (BS) allele used in this study, \( \text{para}^{bss1} \), previously named \( \text{bss}^{1} \), is the most seizure-sensitive of fly mutants, the most difficult to suppress by mutation and by drug, and is a model for human intractable epilepsy (Ganetzky and Wu 1982; Parker et al. 2011a). The \( \text{para}^{bss1} \) allele is a gain-of-function mutation caused by a substitution (L1699F) of a highly conserved residue in the third membrane-spanning segment (S3b) of homology domain IV (Parker et al. 2011a).
study, we use para^{bss1} and para^{bss1/+} as genetic backgrounds to screen for enhancers and suppressors of seizure, respectively. The *cas* gene is located at 14B on the cytological map and encodes an ethanolamine kinase (Pavlidis et al. 1994). The BS allele used in this study is *cas*^{PCR0}, which is caused by a 2-bp deletion that introduces a frame shift; the resulting truncated protein lacks a kinase domain and abolishes all enzymatic activity (Pavlidis et al. 1994). Df(2R)Exel7135=51E2-51E11 contains approximately 22 genes. Df(2R)Exel6056=44A4-44C2 contains approximately 39 genes. Df(2R)Exel6078=58B1-58D1 contains approximately 35 genes. UAS-gishRNAi and other UAS-RNAi lines were obtained from the Vienna Drosophila RNAi Center. All other lines, including Gal4 drivers and deletion lines, were obtained from the Bloomington Drosophila Stock Center.

**Figure 1** Behavior phenotypes for *para^{bss1}* mutants. (A) Illustration depicting stereotype behavioral phenotype of *para^{bss1}* flies subjected to a mechanical shock (10-sec vortex: “bang!”): initial seizure-like behavior, followed by complete paralysis and then a tonic/clonic period that is unique to *para^{bss1}* and not evident in other BS mutant genotypes. One clonus-like event is depicted, but the number can vary, as can the duration of the period. The tonic/clonic-like period is followed by a recovery seizure, and the fly then recovers. Not depicted is a quiescent period of variable duration often observed between the recovery seizure and recovery, as well as the refractory period during which flies are resistant to further seizures that occurs immediately following recovery. (B) Recovery times from behavioral paralysis for *para^{bss1}/Y* hemizygous males (labeled “bss/Y”) is substantially longer than for *para^{bss1/+}* heterozygous females (labeled “bss/+”). For the enhancer screen described in the text, heterozygous deletions were selected that prolonged the *para^{bss1}/Y* recovery time compared to sibling controls. For the suppressor screen described in the text, heterozygous deletions were selected that reduced the percentage of *para^{bss1}/+* females paralyzed by the mechanical shock compared to sibling controls. (Figure adapted from Parker et al. 2011a).

**Behavior and electrophysiology**

Behavioral testing for BS paralysis was performed on flies 2–3 d after eclosion, as described previously (Kuebler and Tanouye 2000). Flies were anesthetized with CO₂ before collection and tested the following day. For testing, 15–20 flies were placed in a food vial and stimulated mechanically with a VWR vortex mixer at maximum speed for 10 sec. For analysis, recovery time was measured for each fly from the end of the vortex stimulation until it resumed an upright standing position. Mean recovery time (MRT) was the average time taken for a fly exhibiting BS behavior to recover in a population. Pools of flies are combined (in total, n = 100 for each genotype). For the purposes of comparisons, these are expressed here as normalized mean recovery time (nMRT), which is the MRT of the experimental flies divided by MRT of their control siblings. For genotypes that display only partial penetrance of BS paralysis, only those flies that displayed paralysis were used for recovery time analysis. A simpler measure of recovery time is RT₅₀ (50% recovery time), the time at which half of BS flies have recovered from paralysis. RT₅₀ was used in some analyses and especially to facilitate initial identification of enhancers and suppressors.

In *vivo* recording of seizure-like neuronal activity and seizure threshold determination in adult flies was performed as described previously (Kuebler and Tanouye 2000; Lee and Wu 2002). Flies 2–3 d posteclosion were mounted in wax on a glass slide, leaving the dorsal head, thorax, and abdomen exposed. Stimulating, recording, and ground metal electrodes were made of uninsulated tungsten. Seizure-like activity was evoked by high-frequency electrical brain stimulation (0.5-ms pulses at 300 Hz for 400 ms) and monitored by dorsal longitudinal muscle recording. During the course of each experiment, the giant fiber circuit was monitored continuously as a proxy for holobrain function. For each genotype tested, n ≈ 10, and unless otherwise noted, all flies were female. Comparisons of paralytic recovery time and seizure threshold were Student *t*-test. For all figures, error bars represent SEM, and statistical significance is indicated by *P < 0.01 and **P < 0.0001.
The parabss1 mutant displays phenotypes that are similar to other mutants of the BS paralytic class such as eab380, sdaio78, and tkor271 (Ganetzky and Wu 1982; Royden et al. 1987; Pavlidis et al. 1994; Zhang et al. 2002), albeit more severe. BS seizure-like behaviors and paralysis are observed in response to mechanical shock (“a bang”) (Figure 1). The time of BS paralysis for parabss1 is much longer than for other mutants and exhibits unusual tonic-clonic-like behaviors. For example, total paralytic time for parabss1 is about 240 sec, longer than for sdaio78, which is about 25 sec (Zhang et al. 2002; Parker et al. 2011a). The parabss1 mutant also has a low threshold for seizure-like activity evoked by high-frequency electrical stimulation (HFS) of the brain. For example, seizure threshold for parabss1 is 3.2 ± 0.6 V HFS, lower than the threshold for sdaio78, which is 6.2 ± 0.8 V HFS; wild-type Canton-Special flies have a seizure threshold of 30.1 ± 3.8 V HFS, for comparison (Figure 2) (Kuebler et al. 2001).

Despite the existing severity of parabss1 phenotypes, we explored the possibility that these might be exacerbated further by enhancer mutations. We have previously found that recovery time from BS paralysis for parabss1 varies with genetic background, age, and other factors (Parker et al. 2011a). The length of time required for recovery appears to be primarily dependent on the number of bouts of tonic-clonic-like activity. We exploited this in an initial screen, investigating the possibility that potential enhancers may reside in chromosomal segments made haploid by deletions, and these would become manifest by a change in the time required to recover from BS paralysis. We then examined enhancers for effects on other parabss1 phenotypes. We measured BS paralytic recovery times in parabss1/Y; Df/+ flies compared with their control siblings of genotype parabss1/Y; Balancer/+ (Table 1, File S1). Several deficiency chromosomes consistently showed increased recovery times for parabss1 males (Table 1). For example, Df(2R)Exel7135 had a MRT of 363 s for experimental males, compared with 234 sec for their sibling controls yielding an nMRT of 1.55. Other notable deficiencies included: Df(2R)Exel6078 and Df(2R)Exel6056 with nMRTs of 2.27 and 2.53, respectively. Here we focus on Df(2R) Exel7135 as representative of our findings on parabss1 enhancers.

**Reduced expression of charlatan (chn) contained in the Df(2R)Exel7135 chromosomal segment enhances parabss1 BS paralysis but not seizure threshold**

The Df(2R)Exel7135 deficiency is a deletion spanning from 51E2 to 51E11 on chromosome 2R and contains approximately 22 genes. Deletion analysis further limited this segment to 51E2 to 51E7 on the basis of observations that the parabss1/Y recovery time is not enhanced by the heterozygous Df(2R)BSC46/+ (51E7-52C2) but is enhanced by Df(2R)BSC651/+ (51C5-51E2) (Figure 3, File S4). We found that BS enhancement in the segment is accounted for by reduced expression of the charlatan (chn) gene. The gene is broken by the 51E2 breakpoints of Df(2R)Exel7135 and Df(2R)BSC651 and is the only apparent gene affected by both rearrangements. Further identification of chn as an enhancer of parabss1/Y is by UAS-chnRNAi. Flies of the genotype ELAV-Gal4F25S parabss1/Y;UAS-chnRNAi/+ show increased BS recovery times with an MRT of 261.9 ± 17.1 sec compared with 105.6 ± 9.4 sec for their ELAV-Gal4 parabss1/Y;+ sibling controls for an nMRT of 2.48 (P < 0.001) (File S4).

The chn gene encodes an NRFS/REST transcriptional repressor of neuronal-specific genes (Escudero et al. 2005; Tsuda et al. 2006; Yamasaki et al. 2011). It has not been previously identified in seizure susceptibility or electrical excitability. Surprisingly, the enhancement of parabss1 by chn was limited to BS paralysis recovery time phenotype, that is, an increase in the severity of this phenotype; there was no apparent enhancement of the other major phenotype: threshold for evoked seizure. For example, flies of the genotype ELAV-Gal4F25S parabss1/Y;UAS-chnRNAi/+ have a seizure threshold of 3.32 ± 0.47 V HFS, similar to the threshold of 3.87 ± 0.53 V HFS (P = 0.46) for their sibling controls (File S3). Flies of the genotypes ELAV-GAL4C355/Y; UAS-chnRNAi/+ and Df(2R)Exel7135/Cyo exhibited no bang sensitivity, indicating that chn enhances seizure severity without being a bang-sensitive mutant itself (File S4). These findings are consistent with the results of Df(2R)Exel7135 and all of the other enhancers identified in this screen: the enhancers increased BS paralysis time to recovery but did not reduce seizure threshold in electrophysiology tests.

**Screening for parabss1 suppressors with deficiencies**

The parabss1 mutant is severely seizure sensitive: phenotypes are difficult to suppress by antiepileptic drug feeding and Drosophila seizure-suppressor mutations thus far identified have been ineffective at alleviating parabss1 phenotypes. The parabss1 mutation is semidominant with seizure-like behaviors, and BS paralysis reduced in heterozygous parabss1/+ flies, but still present at high penetrance (>95%) (Figure 1) (Ganetzky and Wu 1982; Parker et al. 2011a). We exploited this feature to screen for suppressor mutations inferring that heterozygotes would provide a genetic background that is sensitized for detecting putative suppressors. As an initial screen, we investigated the possibility that potential suppressors may reside in chromosomal segments made haploid by deletions and that these would become manifest by a change in BS paralysis. That is, we compared parabss1/+; Df/+ females with their control sisters of genotype parabss1/Y; Balancer/+ for differences in the percentage of flies undergoing BS paralysis. Several deletion chromosomes consistently reduced the BS phenotype in parabss1/+ females (Table 2, File S1). For example, only 13%
of para<sup>baus</sup> females showed BS paralysis compared with their sibling controls, an apparent phenotypic suppression of approximately 87%. Other notable deletions included Df(2R) Excel6285 and Df(3L)ED4502 that caused 97% and 93% suppression, respectively. Here, we focus on Df(3R)ED10639 as representative of our findings on para<sup>baus</sup> suppressors.

**Reduced expression of gilgamesh (gish) contained in the Df(3R)ED10639 chromosomal segment suppresses para<sup>baus</sup> BS paralysis**

The Df(3R)ED10639 deficiency is a deletion spanning from 89B7 to 89D5 and contains approximately 57 genes. In this section, we describe analyses showing that para<sup>baus</sup> suppression in the segment is accounted for by reduced expression of the gilgamesh (gish) gene (Figure 4). para<sup>baus</sup> BS suppression phenotype was mapped to a small region on chromosome 3R between 89B9 and 89B12 using overlapping deficiencies. In particular, localization of the suppression phenotype is based on its inclusion in the Df(3R)Excel7329 deletion, which affects the number of animals paralyzed (Figure 4) (89B9-89B13), and its exclusion from the Df(3R)Excel6269 deletion which has no effect on paralysis (Figure 4) (89B12-B18). This localization is consistent with the combined findings from other overlapping deletions in the region (Figure 4).

The 89B9-89B12 segment contains six genes (Figure 4). We found that an allele of belphegor (bor), para<sup>baus</sup>;bor<sup>05-986</sup>, which showed similar BS paralysis compared with control siblings (9% reduction in BS paralysis), did not appear to cause suppression based on flies of the genotype: Also, an allele of tanaris (tara) did not appear to cause suppression based on flies of the genotype para<sup>baus</sup>;tara<sup>1</sup>, with BS paralysis similar to their sibling controls (0% reduction in BS paralysis). In contrast, an allele of gilgamesh (gish) caused substantial suppression based on flies of the genotype para<sup>baus</sup>;gish<sup>04895</sup>, which showed a 57% reduction in BS paralysis compared with their para<sup>baus</sup>;TM3/+ control siblings (File S4).

**The gish gene**

The gish gene of Drosophila is homologous to mammalian casein kinase CK1g3, both members of the CKI family of serine-threonine kinases (Zhai et al. 1995). The Drosophila gene is approximately 30 kb and alternatively spliced to express 12 different isoforms in four main classes (Hummel et al. 2002; Tan et al. 2010). These arise from two initiation sites: two classes of long transcript (~3 kb) arise from an upstream initiation site; two classes of short transcript (~2.5 kb) from a downstream initiation site (Hummel et al. 2002; Tan et al. 2010). The gish<sup>04895</sup> mutation is a P-element insertion in exon 2, present in long, but not short gish transcripts. Reverse-transcription polymerase chain reaction analysis (Tan et al. 2010) has shown that long gish transcripts are apparently undetectable in gish<sup>04895</sup> mutants. Interestingly, in contrast, short transcripts appear to be more abundant in gish<sup>04895</sup> mutant than in wild-type flies (Tan et al. 2010). In the present experiments, gish<sup>04895</sup> acts as a recessive lethal, in contrast to previous reports, suggesting that it is a viable (Tan et al. 2010).

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**Table 1 Chromosomal deletions that enhance the behavioral bang-sensitive (BS) paralytic phenotype of para<sup>baus</sup>/+ flies**

| Deficiency                          | Experimental (Df) MRT (s) | Control (Balancer) MRT (s) | nMRT |
|------------------------------------|--------------------------|---------------------------|------|
| Df(2R)Excel7135                     | 363                      | 234                       | 1.55 |
| Df(2R)Excel6078                     | 306                      | 135                       | 2.27 |
| Df(2R)Excel7094                     | 232                      | 102                       | 2.27 |
| Df(2R)Excel6071                     | 217                      | 118                       | 1.84 |
| Df(2R)Excel6056                     | 215                      | 85                        | 2.35 |

Values of the length of time that hemizygous para<sup>baus</sup>/Y males remained paralyzed are depicted as MRT. To minimize the effects of genetic background, experimental males of the general genotype: para<sup>baus</sup>/Y;Df/+ were compared directly with sibling control brothers arising from the same cross (genotype: para<sup>baus</sup>/Y;Balancer/+). The ratio of MRT for experimental males with that of their control siblings is listed as nMRT. MRT, mean recovery time; nMRT, normalized mean recovery time.
We are unclear on the reasons for this apparent difference in viability. We find that precise excision of the gish04895 P-element completely reverted the BS suppressor phenotype (Figure 4, File S2, File S4), restored viability, but did not appear to revert the male sterility phenotype seen among gish mutant alleles (Castillón et al. 1993).

Identification of gish as a parabss1/+ BS suppressor by mutant analysis was supported further by RNAi analysis. Flies of the genotype ELAV-Gal4C155 parabss1/++;UAS-gishRNAi/+ showed a 75% reduction in BS paralysis compared with their ELAV-Gal4C155 parabss1/+;+/+ control siblings, showing that BS suppression occurred when gish expression was reduced in all neurons with the ELAV-Gal4 pan-neuronal driver (File S4). We propose that gish is a suppressor of parabss1/+ based on reversion of phenotypes by gish04895/+, by ELAV-Gal4C155, driven UAS-gishRNAi, by Df(3R)ED10639/+, and by Df(3R)Exel7329/+. Several mutant alleles of gish that failed to suppress parabss1/+ BS paralytic phenotypes were also found in these analyses. Thus, suppression was not observed for 3 P-element mutations with inserts in the second intron of gish which is spliced out of the long transcripts (genotypes: parabss1/+;gish08891, parabss1/+;gish09641, and parabss1/++;gish10667, Figure 4). No suppression was seen in parabss1/+;gish01709/+ flies, which has an insert upstream of the first transcript initiation site (Figure 4, File S4).

The gish04895 mutation raises the threshold for evoked seizures in parabss1/+ flies

The mutation gish04895 is a recessive lethal. As a heterozygote, in a wild-type background, it displays a seizure-resistant phenotype. Thus, the seizure threshold of gish04895/+ flies is about twice that of wild-type Canton-Special flies, 63.4 ± 5.8 V HFS and 33.8 ± 3.2 V HFS, respectively (Figure 5). The gish04895/+ flies have no other apparent phenotypes: their electrophysiology, behavior, and morphology are all wild type.

Seizure-suppression for gish is seen with flies of the genotype: parabss1/+; gish04895/+, which show a seizure threshold of 15.6 ± 2.42 V HFS, which is greater than the threshold of their parabss1/+;TM6/+ control siblings (9.8 ± 1.09 V HFS; Figure 5). This seizure-suppression is caused by a loss of gish function as seen most clearly in deletion flies: parabss1/++;Df(3R)ED10639/+ show a seizure threshold nearly in the wild-type range (22.0 ± 2.62 V HFS; Figure 5).

### DISCUSSION

In the present article, we examine severe seizure phenotypes and explore the possibility that severity may be modulated by genetics. We use as substrate the Drosophila parabss1 mutation a channelopathy affecting the voltage-gated Na+ channel. Severe seizure sensitivity is observed in parabss1 mutants, severity that is unresponsive to available drug treatment. In addition, parabss1 has not responded to seizure suppressor mutations identified in screens based on the Drosophila mutants eas and sda. The present study is based on an unbiased, forward genetics screen for mutations that interact with parabss1 by either exacerbating seizure phenotypes (seizure enhancer mutations) or reducing the severity of phenotypes (seizure suppressor mutations).

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**Table 2 Chromosomal deletions that revert the behavioral bang-sensitive (BS) paralytic phenotype of parabss1/+ flies**

| Deficiency | BS |
|-----------|----|
| Wild type | 0.00 |
| Df(2R)Exel6285 | 0.03 |
| Df(3L)ED4502 | 0.07 |
| Df(3R)ED10639 | 0.13 |
| Df(3L)ED224 | 0.19 |
| Df(3L)ED201 | 0.29 |
| Df(3L)ED4502 | 0.42 |
| Df(3R)BC247 | 0.49 |
| Df(3R)ED5518 | 0.50 |
| Df(3L)ED4486 | 0.50 |
| parabss1/+ | 0.95 |

Ordinarily, approximately 95% of parabss1/+ flies show a BS paralytic phenotype: paralysis after mechanical stimulation. Wild-type flies never show BS paralysis. The number of flies showing BS paralysis is greatly reduced by the deficiency chromosomes listed in the table. Flies tested carried the heterozygous deficiency and were of the general genotype: parabss1/++; Df(3R)Exel7329/+. In all cases, to control for genetic background, experimental flies were compared directly with sibling control flies arising from the same cross (genotype: parabss1/+; Balance/+).

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We tested for gish04895 suppression against BS mutant, eas; gish was ineffective as a suppressor. Thus, eas mutants showed 100% BS paralysis in a gish04895/ background; electrophysiology also showed minimal increases in seizure threshold (Figure 6, File S3, File S4). We also find that gish/+ does not suppress phenotypes of parabss1 homozygous females and parabss1/Y hemizygous males. Thus, parabss1 homozygotes and hemizygotes showed 100% BS paralysis in a gish background: BS paralysis could not be suppressed by gish04895/+, by Df(3R)ED10639/+, or by UAS-gishRNAi. In addition, a Df(3R)ED10639/+ background caused no reductions of BS paralytic recovery time in parabss1 homozygotes and hemizygotes, a phenotype of parabss1 that is extraordinarily easier to suppress than BS paralysis (Figure 6, File S4).

Seizure suppression by gish does not appear to be dependent on Wg/Wnt signaling

The prickle gene functions in noncanonical Wg/Wnt signaling, and mutations have been found to cause myoclonic seizures in humans and BS paralytic behavior in Drosophila (Tao et al. 2011). CK1g caspase kinases subserve a large number of cellular processes with diverse substrates (Knippschild et al. 2003), and one prominent role for gish is to phosphorylate arrow, a co-receptor for Wg (Zhang et al. 2006). To test whether seizure suppression by gish might be via Wg signaling, we examined other components of the pathway by RNAi. To test arrow loss-of-function, flies of the genotype ELAV-Gal4C155 parabss1/++; UAS-arrRNAi/+ showed a slightly lower, but not significant percentage of BS paralysis compared with control ELAV-Gal4C155 parabss1/+;+/+ flies (data not shown, File S4). To test Wg and pangolin loss-of-function, flies of the genotypes ELAV-Gal4C155 parabss1/++; UAS-WgRNAi/+ and ELAV-Gal4C155 parabss1/++; UAS-pangRNAi/+ were comparatively equal in percentage of BS paralysis as their ELAV-Gal4C155 parabss1/++;+ TM6 controls (data not shown, File S4). Thus, we conclude that seizure suppression by gish is not directly linked to Wg/Wnt signaling.
The search for parabi enhancers and suppressors identified several candidates. Analysis of chn was representative of an enhancer. We found that the time of paralysis of parabi individuals was increased (the phenotype screened for), but there was otherwise no obvious enhancement of seizure-sensitivity or severity. Behavioral phenotypes of parabi generally resemble those of other BS mutants: all BS mutants are behaviorally similar in initial seizure, initial paralysis, and recovery seizure (Parker et al. 2011a). Unlike other BS mutants, initial paralysis in parabi homozygotes is followed by an extended period of tonic/clonic-like activity, resembling activity observed in several human epilepsies (Parker et al. 2011a). During this period in parabi, the fly is mainly quiescent, resembling a tonic phase. The quiescence is broken up by multiple bouts of clonus-like activity. Because of its period of tonic/clonic-like activity, bs1 recovery time is much longer than for other BS mutants such as sda or eas (Parker et al. 2011a). It is this recovery time, the tonic/clonic period, that is extended by the chn enhancer mutation. A surprise to us was that there was no chn enhancement of the other major parabi phenotype-a low electrophysiology seizure threshold. Also, the chn mutation is the only seizure enhancer that we have identified thus far, that does not cause any BS phenotypes (Glasscock and Tanouye 2005; Heimatscfe et al. 2006).

Analysis of gish was representative of a parabi suppressor. We found that seizure sensitivity of heterozygous parabi/+ individuals was greatly reduced by gish loss-of-function mutation and by RNAi. Also, electrophysiological threshold is increased, a further indication that seizure-susceptibility has been reduced in parabi/+. Previously, we have identified 13 seizure-suppressor mutations that suppress the BS behavioral phenotypes of sda and eas mutants, and raise the electrophysiology seizure threshold, often to nearly wild-type levels (reviewed in Parker et al. 2011b). However, seizure suppressors identified heretofore have been ineffective at suppressing parabi phenotypes. Seizure suppression by gish loss-of-function mutations reported here is unusual in several respects. It is the only seizure suppression that is effective in reverting parabi phenotypes, although...
it is effective only with heterozygotes, and not homozygotes or hemizygo
tes. Surprisingly, the seizure suppression is ineffective with sda and eas mutants. Previously, we had attributed this simply to dif
erent seizure-sensitive mutants being more or less refractory to sup-
pression. The present results suggest, however, that there may be a
fundamental difference between sda and eas mutants, on the one
hand, and parabss1 on the other. The nature of the difference remains
unclear, at present, but parabss1 seems somehow to be special. We
suspect that this could be because of something special about the
voltage-gated Na+ channel, the gain-of-function nature of the par-
abss1 mutation, or both. Also, somewhat perplexing is the reason why
Figure 5 Suppression of sei-
zure threshold by gish04895
and Df Ed10639. Seizure-like
activity was recorded in flies of
different genotypes. Depicted
are the relative HFS voltages
required to evoke seizure-like
activity at threshold. Loss-of-
function mutations of gish suppress seizure-sensitivity in parabss1 heterozygotes, indicated by an increase in seizure
threshold voltage compared to
controls. In each case, experi-
mental flies are compared with
controls that are siblings arising
from the same cross in order
to minimize genetic back-
ground differences. (A) Seizure
threshold of gish04895/+ com-
pared with the wild type. The
heterozygous mutant gish04895/+ has a slightly greater voltage at
threshold suggesting that it is a
seizure-resistant mutation. (B) Seizure thresholds of parabss1
heterozygotes in different sei-
zure-suppressor backgrounds.
Experimental gish04895/+ flies
were of the genotype parabss1/+;
gish04895/+ and had a greater sei-
zure threshold than their control sib-
lings (genotype: parabss1/+; TM6,
Df/+), indicating seizure-suppression. Experimental Df Ed10639/+ flies
were of the genotype parabss1+/
; Df(3R)Ed10639/+ and had a
greater seizure threshold than
their control siblings (genotype:
parabss1/+; TM3/+ indicating
seizure suppression. Experimen-
tal ELAV-Gal4-driven gishRNAi flies were of the genotype
ELAV-Gal4C155; parabss1/+;
UAS-gishRNAi/+ and had a higher sei-
zeure threshold than their control
siblings (genotype: ELAV
Gal4C155; parabss1/+; TM6/+)
indicating seizure-suppression.

It is clear from this study that gish is capable of suppressing
parabss1/+ phenotypes and from other deletions identified in our
screen that additional suppressor mutations may be found. The parabss1 mutant has been presented as a model for human intractable epilepsy,
especially Dravet syndrome (Dravet 1978), a Na+ channelopathy
(Parker et al. 2011a). The findings presented here on gish suppression
of parabss1 suggest a compelling novel approach for developing options
for intractable epilepsy therapeutics depending on exactly how well
parabss1 models Dravet syndrome or other intractable epilepsies

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between parabss1 present, available data show that the paralytic behavioral phenotype is not reduced by 100% of altered by seizure disorders, especially intractable disorders. Suppressors may bring us closer to unraveling the complexity of gish04895 trol siblings (genotype: fl e si nb o t he x p e r i m e n a l( g e n o t y p e : gish04895; fl eas) genotypes. (A) The percentage of heterozygotes. (A) The percentage of
(B) Electrophysiological recording shows that the seizure threshold of eas is a little greater in a gish04895/+ background (genotype: eas; gish04895/+), but there is no significant suppression compared with control siblings (genotype: eas; TM6, Dr/+). (C) Recovery time of parabss1 homzygotes and hemizygotes is not altered by gish loss-of-function. Depicted are recovery times compared between parabss1; Df(3R)Ed10639/+ experimental flies and their control siblings (genotype: parabss1; TM3/+

Figure 6 Suppression of seizure sensitivity by gish is specific to parabss1 heterozygotes. (A) The percentage of eas flies showing a bang sensitive paralytic behavioral phenotype is not reduced by gish04895/+. Paralysis is 100% of flies in both experimental (genotype: eas; gish04895/+) and control siblings (genotype: eas; TM6, Dr/+ ) genotypes. (B) Electrophysiological recording shows that the seizure threshold of eas is a little greater in a gish04895/+ background (genotype: eas; gish04895/+), but there is no significant suppression compared with control siblings (genotype: eas; TM6, Dr/+ ). (C) Recovery time of parabss1 homzygotes and hemizygotes is not altered by gish loss-of-function. Depicted are recovery times compared between parabss1; Df(3R)Ed10639/+ experimental flies and their control siblings (genotype: parabss1; TM3/+).

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