Neurobiology of Disease

Seizure Suppression by *top1* Mutations in *Drosophila*

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DNA topoisomerase I is an essential nuclear enzyme involved in resolving the torsional stress associated with DNA replication, transcription, and chromatin condensation. Here we report the discovery of a seizure-suppressor mutant, *top1*^{JS}, which suppresses seizures in a *Drosophila* model of human epilepsy. A P-element mutagenesis screen using *easily shocked* seizure-sensitive mutant as a genetic background identified *top1*^{JS}, which plays a novel role in regulating nervous system excitability. Plasmid rescue, excision, complementation, and sequencing analyses verified that *top1*^{JS} results from a P-element insertion in the 5′ untranslated region. Quantitative reverse transcription analysis on wild-type and mutant fly heads showed that the *top1*^{JS} mutation causes reduced transcription level in the CNS, suggesting a partial loss-of-function mutation. Electrophysiological experiments revealed normal seizure thresholds in *top1*^{JS} mutants, which are different from other seizure suppressors identified previously, suggesting a novel mechanism underlying seizure suppression by *top1*^{JS}. The pharmacological camptothecin feeding experiment and cell death analysis suggested that the seizure suppression by *top1*^{JS} may occur via increased neuronal apoptosis. Furthermore, overexpression of the *DIAP1* (*Drosophila* inhibitor of apoptosis 1) gene rescues *top1*^{JS} suppression, providing additional support for a neural apoptosis suppression mechanism. The *top1*^{JS} mutation is the first viable partial loss-of-function mutation identified in higher eukaryotes, and the results presented here point to a novel function for topo I in construction and/or maintenance of circuits required for seizure propagation *in vivo*.

Key words: **topoisomerase I; seizure; suppression; camptothecin; epilepsy; *Drosophila***

Introduction

Eukaryotic DNA topoisomerases are ubiquitous and essential nuclear enzymes involved in various DNA transactions (Wang, 1996, 2002; Champoux, 2001). DNA topoisomerases are classified as type I and type II; they differ structurally and mechanistically (Champoux, 2001). Eukaryotic type I topoisomerase (topo IB) plays important roles in DNA replication, RNA transcription, DNA recombination, chromosome condensation, and the maintenance of genomic stability (Champoux, 2001; Wang, 2002). Immunohistochemical analysis in selected mouse brain regions reveals that a significant level of topo I activity is present in the brain, and the level of topo I activity varies among different brain regions (Plaschkes et al., 2005). The cerebellum, visual cortex, and the striatum exhibit higher topo I activity than the hippocampus and hypothalamus. Dual-label immunofluorescence analysis reveals that topo I-expressing neurons are both inhibitory and noninhibitory neurons (Plaschkes et al., 2005).

Mammalian DNA topoisomerases are the targets of several anticancer drugs used today in the clinic, including the topo I inhibitor camptothecin (CPT) and its derivatives (Wang, 1994; Wang et al., 1997; Pommier, 1998; Pommier et al., 1999; Li and Liu, 2000). Several *in vitro* studies have shown that CPT induces apoptosis in cultured neurons (Morris and Geller, 1996; Park et al., 1997). Although apoptotic cell death occurs physiologically during development, neuronal apoptosis is also associated with various neurodegenerative disorders and conditions of neural trauma including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, AIDS dementia, epilepsy, retinitis pigmentosa, cerebellar degeneration, and ischemic brain injury and stroke (Thompson, 1995). It is not known how these anticancer agents signal a postmitotic neuron to execute its apoptotic pathway.

The work presented here describes a mutant screen that allows us to identify novel loss-of-function seizure suppressors by P-element mutagenesis. Seizure-suppressor genes are identified by mutations that revert the seizure-sensitive phenotype in a *Drosophila* model of epilepsy (Kuebler et al., 2001). Seizure-suppressor mutants were isolated by mobilizing a P element in an *easily shocked* (*eas*) mutant background and looking for second-site mutations that reduce seizure susceptibility. Of multiple seizure-suppressor genes identified, we focus on *top1*^{JS}, a new allele of the gene *top1* that has not been previously implicated in seizure susceptibility. The *top1* gene has been primarily associated with growth and development in *Drosophila* (Lee et al., 1993). Mutants of *top1* exhibit abnormal proliferation and defective nuclear morphology in ovary cells: there are extranumeral germ-line cells in individual egg chambers, and the follicle cells are underreplicated (Zhang et al., 2000). Topo I is maternally stored in embryos, and embryos from mutant mothers frequently show disrupted nuclear divisions with defects in chromosome

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condensation and segregation. Topo I localizes to the nuclei during interphase and prophase but disperses into the cytoplasm at metaphase. The cytological and genetic analyses of the *top1* mutants suggest that topo I plays critical roles in many developmental stages active in cell proliferation.

We report a surprising new role for topo I in the nervous system as a seizure suppressor. The *top1* mutation $top1^{JS}$ isolated in this study acts as a general seizure suppressor, reducing the seizure susceptibility of *eas*, *slamdance* (*sda*), and *bang-senseless* (*bss*) seizure-sensitive flies. Plasmid rescue, P-element excision, complementation, and sequence analyses verified that $top1^{JS}$ is a new allele of the *top1* gene resulting from a P-element insertion in the 5′ untranslated region (UTR). Reverse transcription (RT) analysis on wild-type and mutant fly heads showed that the $top1^{JS}$ mutation causes reduced transcription level in the CNS, suggesting a partial loss-of-function mutation. Electrophysiological experiments revealed normal seizure thresholds in the $top1^{JS}$ mutation that are different from other seizure suppressors identified previously, suggesting a novel mechanism underlying seizure suppression. Pharmacological experiment and cell death analysis suggested that the seizure suppression by $top1^{JS}$ may occur via the increased neuronal apoptosis. Overexpression of the *Drosophila* inhibitor of apoptosis 1 (*DIAP1*) gene rescues $top1^{JS}$ suppression, providing additional support for a neural apoptosis suppression mechanism. Finally, we discussed the potential practical implications based on genetic and drug-feeding experiments.

Materials and Methods

Fly stocks. Drosophila strains were maintained on standard cornmeal–molasses agar medium at room temperature (22–25°C). The *eas* gene is located at 14B on the cytological map and encodes an ethanolamine kinase (Pavlidis et al., 1994). The *eas* allele used in this study is eas^{PC80}, 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). The bang-sensitive (BS) phenotype of *eas* flies is completely penetrant; thus, in this study, we use *eas* BS mutation as a genetic background to screen its suppressors by P-element hybrid dysgenesis. The *sda* gene is located at 97D on the third chromosome and encodes an aminopeptidase N. The allele used in this study is $sda^{iso7.8}$, which is caused by a 2-bp insertion in the 5′ untranslated region; phenotypes appear to be a result of enzyme underproduction (Zhang et al., 2002). The single P-element stock used in this screen is *y w P{lacw}*3–76a, which contains a single *P{lacw}* insertion at the cytological position of 18C-D. The topo I alleles used in this study are as follows: $top1^{G0229}$, $top1^{G0134}$, $top1^{G0201}$, $top1^{77}$, and $top1^{112}$. All alleles are lethal and behave genetically as nulls, indicating that the *top1* gene is essential for viability. To figure out the exact insertion sites of $top1^{G0229}$, $top1^{G0134}$, and $top1^{G0201}$ alleles, plasmid rescue was performed on these three alleles. Results showed that $top1^{G0229}$ allele is caused by a *P{lacw}* insertion in the first intron of *top1* at the scaffold position 257493/257494; the $top1^{G0134}$ allele is caused by a *P{lacw}* insertion in the second exon of *top1* at the scaffold position 257709/257710; and the $top1^{G0229}$ allele is caused by a *P{lacw}* insertion in the fourth intron of *top1* at the scaffold position 260024/260025 (Fig. 1). In addition, one previously studied allele, $top1^{112}$, is attributable to a deletion in the 3′ untranslated region of *top1* (Lee et al., 1993; Zhang et al., 1996). The deficiency stock used in this work is *Df(1)ED7294*, which has a deletion in the region between 13B1 and 13C3. The *top1* rescue construct used here is *P{top1 ± 10}*, which contains a 10 kb genomic *Spe*I–*Bam*HI fragment. This 10 kb genomic fragment includes the entire *top1* transcribed region and 1.3 kb of upstream and 0.5 kb of downstream sequences; it contains no complete gene other than the 8.5 kb *top1* gene (Lee et al., 1993) (http://flybase.bio.indiana.edu). All above fly stocks were obtained from Bloomington Stock Center at Indiana University. The transgenic upstream activator sequence (UAS)–DIAP1 line was obtained from Dr. John Nambu (University of Massachusetts, Amherst, Amherst, MA).

Figure 1. The *top1* locus at 13B6 on chromosome X, scaffold AE003498. The *top* locus spans 8.5 kb and encodes three different transcripts: top1-RA (5325 nt), top1-RB (2379 nt), and top1-RC (4940 nt). For simplicity, only the transcript corresponding to top1-RA is shown. The exons are represented as numbered boxes. The gray areas correspond to untranslated regions (including exon 2–7, part of exon 1, and part of exon 8), whereas the black areas correspond to protein-coding regions. Alleles of $top1^{G0229}$, $top1^{G0134}$, and $top1^{G0201}$ are P-element insertion lines. The $top1^{112}$ allele has a deletion in 3′ untranslated region. The P element responsible for the $top1^{JS}$ mutation is inserted in the 5′ untranslated region, between nucleotides 256075 and 256076 of genomic scaffold AE003498, corresponding to 31 and 32 of the *top1* mRNA variant A and C, which is 257 bp away from the translation start site.

P-element mutagenesis screen scheme and behavioral tests. Female *y w eas P*[*lacw*] flies were crossed to males *w; Sp/CyO*, δ *2, 3 Dr/TM3 Sb*, and δ 2, 3 male progeny were crossed to virgin C(1)DX females. The male flies were tested for BS behavior: BS flies were discarded, but exceptional bang-resistant (BR) flies were kept for additional analysis. By using this P-element mutagenesis screening scheme, X chromosomal recessive and dominant suppressors and autosomal-dominant suppressors can be isolated. Of 26,833 male flies screened, 34 suppressors were isolated from behavior testing. Seizure suppression varied from 90% (strongest) to 10% (weakest); $top1^{JS}$ suppressed *eas* by ~63%.

BS behavioral tests were performed on flies 2–3 d after eclosion. Flies were anesthetized with CO_2 before collection and tested the following day to eliminate any CO_2 effects on behavior. For testing, 15–20 flies were placed in a food vial and stimulated with a VWR Vortex mixer (VWR International, West Chester, PA) at maximum speed for 10 s. BS paralytic mutants undergo seizures characterized by brief hyperactivity (2 s) and temporary paralysis (20–300 s). Recovery times were calculated by measuring the time from the end of the vortex until the flies resumed an upright standing position.

Electrophysiology. All flies were used for electrophysiology 2–3 d after eclosion. Flies were mounted for electrophysiology without anesthesia by capturing and immobilizing them with a vacuum line. Immobilized flies were then attached to a pin glued to the dorsal thorax with Super Glue gel (Scotch; 3M, St. Paul, MN). Two uninsulated tungsten electrodes were inserted into the brain for stimulation. The ground electrode was inserted into the abdomen. Recording electrodes were uninsulated tungsten electrodes inserted into the dorsal longitudinal muscles (DLMs) or tergotrochanter muscles identified by their thoracic insertion sites (Tanouye and Wyman, 1980). Two types of electrical stimulation were used: single-pulse stimulation and high-frequency (HF) stimulation. Single-pulse stimuli (0.2 ms duration, 0.5 Hz) were delivered to the brain to drive the giant fiber (GF). During the course of each experiment, the GF was stimulated continuously with single-pulse stimuli to assess GF circuit function. HF stimulation (0.5 ms pulses at 200 Hz for 300 ms) was used to elicit seizures. Seizure-like activity in *Drosophila* is observed as uncontrolled, high-frequency (>100 Hz) neuronal firing, most conveniently examined in motoneurons. Seizures are extensive: >30 motoneurons innervate at least seven different thoracic muscle groups participate in seizures (Kuebler and Tanouye, 2000). To determine seizure threshold, HF stimuli were initially given to flies at predicted intensities, depending on their genotypes. If the stimulus fails to elicit a seizure, the intensity was subsequently increased at 1 V increments until a seizure was induced. The threshold was determined for an individual fly as the lowest

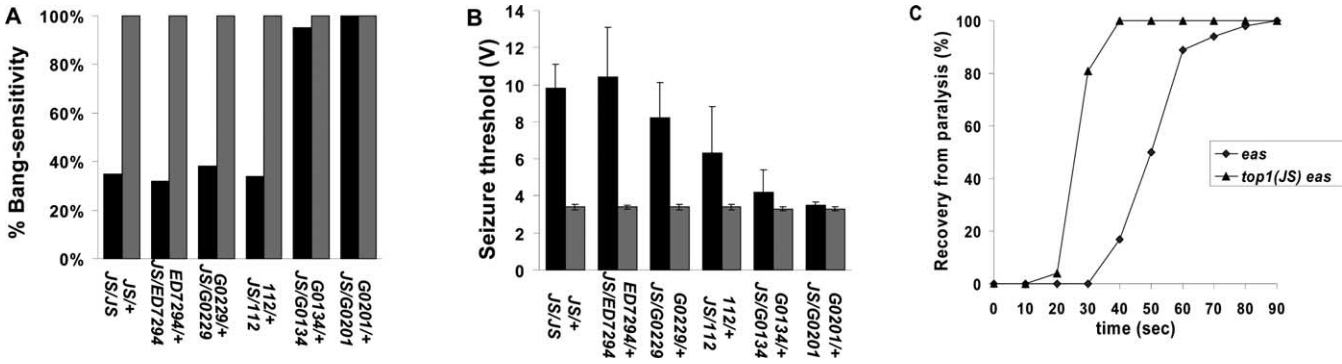

Figure 2. Behavioral bang sensitivity and seizure threshold in heteroallelic combination of *top1^{JS}* and various other *top1* mutants in an *eas* mutant background. The data here reveal that combinations of *top1^{JS}* allele with different *top1* alleles display different suppression efficiency in the *eas* mutant background. ***A***, Behavioral bang sensitivity in various *top1* allelic combinations. ***B***, Seizure thresholds in various *top1* allelic combinations. Seizure thresholds are consistent with behavioral testing. Black bars represent the behavioral bang sensitivity and seizure thresholds in different *top1* allelic combinations with *top1^{JS}* in an *eas* background. Gray bars represent heterozygous *top1* alleles in an *eas* background; they act as controls in this assay. ***C***, Comparison of recovery time in *eas* and *top1^{JS} eas* double mutants. Shown is the percentage of flies recovered with time and a cumulative measure that includes the initial behavior seizure, the paralysis period, and the recovery seizure, which are not indicated separately. The number of flies standing at intervals after the shock was recorded until the entire population had recovered. For each genotype, $n \geq 80$ flies tested for behavior; $n \geq 8$ flies tested for electrophysiology; $n \geq 30$ flies recorded for recovery time. For behavioral bang sensitivity and seizure threshold, results from the allelic combinations of *top1^{JS}/top1^{JS}*, *top1^{JS}/Df (1)ED7294*, *top1^{JS}/top1^{G0229}*, and *top1^{JS}/top1^{112}* are statistically different from their corresponding controls ($p < 0.05$), whereas results from the allelic combinations of *top1^{JS}/top1^{G0134}* and *top1^{JS}/top1^{G0201}* are not statistically different from their corresponding controls ($p > 0.05$).

intensity at which seizures occurred. The fly was allowed to rest 15 min after each HF stimulation.

Molecular mapping of top1 ^{JS}. The combination of plasmid rescue and sequencing approaches was used to determine the exact P-element insertion site of *top1^{JS}*. The plasmid rescue was performed as described previously with some modifications (Wilson et al., 1989). *Drosophila* genomic DNA was isolated as described on the Berkeley Drosophila Genome Project website (http://www.fruitfly.org/about/methods/inverse.pcr.html). Genomic DNA (2–5 μg) was digested with *Eco*RI, and the fragments were self-ligated with T4 DNA ligase. Ligated products were transformed into DH5α electrocompetent cells, and the transformants were selected on ampicillin plates (100 mg/ml). Plasmid DNA from positive clones was isolated and digested with *Eco*RI and *Bam*HI to find different digestion patterns. Plasmid DNA was sequenced and aligned to the genome database [National Center for Biotechnology Information (NCBI) blast program]. The approximate insertion site was determined using a primer near the end of the P-element sequence (CGACGGGACCACCTTATGTTATTTCATCATG). The exact insertion site was then determined by sequencing back toward the P element using a primer specific for the flanking genomic region of *top1* (TCCCTGCTTCACACCATCCTTC).

RT-PCR analysis. Adult wild-type Canton Special (CS) and *top1^{JS}* fly heads were decapitated using a razor, and fly heads were collected in 1.5 ml centrifuge tubes. Total RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA), and 2.5 μg was reverse transcribed using Sprint PowerScript PrePrimed Single Shot (random hexamer primers; Clontech, Mountain View, CA). The primer pair used to amplify *top1* transcripts generated a 499 bp product. Their sequences are as follows: forward, 5′-AGTCGCGACAAGGATCGACACA-3′ and reverse, 5′CGGCAACGTTTCCATTGCCAT-3′. The internal control was fly actin gene CG7478-PA (Act79B) amplified with the primer pair to amplify the actin gene: forward, 5′-ATCCAGGTATCGCTGACCGTATGC-3′ and reverse, 5′-AAAGAAGGCGTTGCCGCTCGTTTC-3′. This primer pair generated a 435 bp gene product. The cDNA products from reverse transcription were serially diluted for sensitivity. PCRs were performed with *Taq*DNA polymerase (Qiagen, Valencia, CA) in a reaction volume of 50 μl. The PCR was performed in a thermal cycler with the following cycling parameters: 2 min at 94°C and then 28 cycles consisting of 30 s at 94°C, 30 s at 58°C, and 30 s at 72°C; after completion of the last cycle, a final incubation at 72°C for 7 min was performed. The PCRs were analyzed on a 1.5% agarose gel. The RT-PCR products were quantified using ImageJ 1.36 (http://rsb.info.nih.gov/ij). The relative *top1* transcript levels in the test and control lanes were determined by normalizing the signal intensity of the *top1* band to that of the corresponding *Act79B* band.

Drug feeding. The topoisomerase I inhibitor CPT (C9911; Sigma, St. Louis, MO) was used. CPT feeding solutions contained 5% sucrose to encourage flies to ingest drug and were mixed with formula 4–24 Blue *Drosophila* medium (Carolina Biological Supply, Burlington, NC) in a 1:1 ratio of dry food to drug solution to create the desired feeding medium. For example, to make 4 μM CPT feeding medium, a 1:1 blend of 8 μM CPT solution with dry food would be made. Progeny were reared in the CPT-containing food and tested at 2–3 d after eclosion. Different BS mutants tolerated drug differently: pupal lethality was observed at different CPT concentrations for different BS mutants; *eas* mutants could tolerate higher concentration CPT than *sda* and *bss* mutants.

Apoptosis analysis. Heads from 2-d-old wild-type and *top1^{JS}* mutant flies were collected and frozen in OCT embedding medium (Tissue Tek; Miles, Elkhart, IN). Cryostat sections (10 μm) were collected on positively charged slides (Fisherbrand Superfrost Plus; Fisher Scientific, Houston, TX) and fixed in 2% glutaraldehyde in PBS (freshly prepared) for 20 min at room temperature. Slides were then washed with PBS and incubated in freshly prepared permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate) for 2 min on ice. Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick end labeling (TUNEL) assay was performed using *In Situ* Cell Death Detection Kit, Fluorescein (Roche Diagnostics, Indianapolis, IN) according to manufacturer's directions. Slides was washed with PBS to stop the reaction and then imaged by confocal microscope.

For agarose gel electrophoresis, genomic DNA samples from wild-type, *eas top1^{JS}* double-mutant, and *top1^{JS}* mutant flies were electrophoretically separated on 1.5% agarose gel containing ethidium bromide (0.5 μg/ml). DNA was visualized by UV transillumination.

Results

Isolation and molecular basis of the *top1^{JS}* mutation

In a screen for new seizure suppressors, the *top1^{JS}* mutation was found to reduce the behavioral paralysis of *eas* flies after a mechanical shock by ~63% and decrease the seizure susceptibility to an electrical shock by three to four times. The *top1^{JS}* mutation was mapped by both molecular and genetic methods. Molecularly, plasmid rescue was used to isolate genomic DNA flanking the insertion site of the P element responsible for *top1^{JS}*. The flanking DNA was then sequenced and compared with the information available in the *Drosophila* genome database using the NCBI blast program. This comparison identified the location of the *top1^{JS}* insertion within the *top1* gene in the 13B6 region of the X chromosome (Fig. 1). By sequencing the flanking genomic DNA back

toward the P element, the exact location of the insertion was identified. The P element responsible for the *top1*^{JS} mutation is inserted in the 5′ untranslated region between nucleotides 256075 and 256076 of the genomic scaffold AE003498, corresponding to 31 and 32 of the *top1* mRNA variants A and C; 257 bp away from the translation start site.

The top1^{JS} mutation raises seizure thresholds and shortens the recovery time of *eas* flies

Drosophila BS paralytics form a class of behavioral mutants with enhanced seizure-sensitive phenotypes. The class includes the following: *bss*, *bas* (*bang-sensitive*), *eas* (ethanolamine kinase) (Pavlidis et al., 1994), *tko* (*technical knockout*) (mitochondrial ribosomal protein) (Royden et al., 1987), *kdn* (*knockdown*) (citrate synthase) (Fergestad et al., 2006), and *sda* (aminopeptidase) (Zhang et al., 2002). All of these BS mutants have been shown to have low seizure thresholds compared with wild-type flies in electrophysiological experiments (Kuebler and Tanouye, 2000; Kuebler et al., 2001). Among BS mutations, *eas* is considered intermediate in terms of both the degree to which it reduces seizure threshold and the ease with which it can be suppressed by second-site modifiers (Kuebler and Tanouye, 2000; Kuebler et al., 2001). We examined the seizure thresholds of suppressors in an *eas* background to see whether double mutants have higher seizure thresholds.

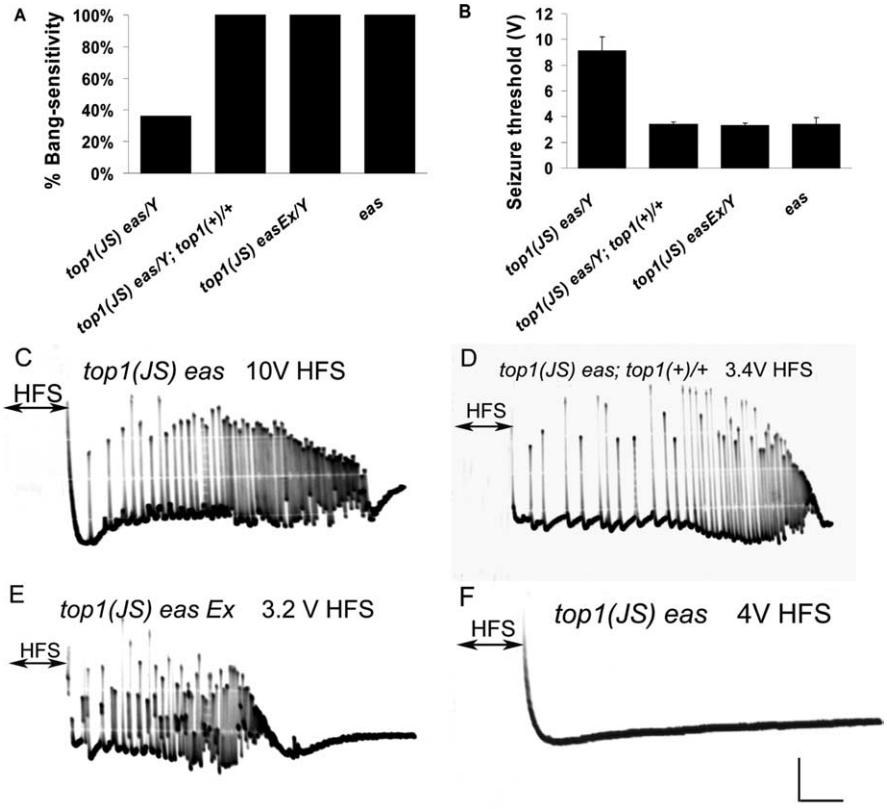

Figure 3. Behavioral and electrophysiological evidences that *top1*^{JS} causes seizure suppression. ***A***, Behavioral evidence that seizure suppression is caused by *top1*^{JS}. The *top1* rescue construct (*top1*+) used here is *P {top1 ± 10}*, which contains the entire *top1* transcribed region and no other complete genes. In the presence of *top1*+, *top1*^{JS} *eas* double mutants show 100% bang sensitivity, which is the same as *eas* mutants (100% BS; $p < 0.01$), suggesting the rescue of the seizure suppression phenotype. The *top1*^{JS} *easEx* is a precise excision line in which the P element is precisely removed, and the behavioral phenotypes of those flies get reverted to 100% bang sensitivity ($p < 0.01$). ***B***, Electrophysiological evidence that seizure suppression is caused by *top1*^{JS}. Seizure thresholds are consistent with the behavioral phenotypes: both rescue and excision lines have decreased seizure thresholds, similar to the *eas* mutant ($p < 0.01$). ***C***, A representative seizure in a *top1*^{JS} *eas* fly recorded from the DLM after a high-frequency brain stimulus (HFS) of 10 V. The DLM shows aberrant high-frequency firing during the seizure phase. ***D***, A seizure is elicited in a *top1*^{JS} *eas; top1(+)/+* fly after a 3.4 V HFS. ***E***, A seizure is elicited in a *top1*^{JS} *easEx* fly after a 3.2 V HFS. ***F***, Failure to elicit seizure in a *top1*^{JS} *eas* double-mutant fly after a 3.4 V HFS. Calibration: 10 mV, 200 ms. For each genotype, $n \geq 80$ flies tested for behavior; $n \geq 8$ flies tested for electrophysiology.

To further investigate a role for *top1* in seizure suppression, we examined additional *top1* mutants in an *eas* background. All of the *top1* alleles studied are X-chromosomal lethals necessitating examination in heteroallelic combinations with *top1*^{JS}. Interestingly, *top1*^{JS} complemented the lethality of all lethal *top1* alleles. However, some, but not all, lethal alleles acted to suppress seizures (Fig. 2*A*,*B*). Thus, *top1*^{G0229} acts to suppress seizures as indicated by the genotype: *top1*^{G0229} *eas/top1*^{JS} *eas* that showed 63% suppression of the behavioral paralytic phenotype compared with their *top1*^{G0229} *eas/+ eas* controls. In addition, electrophysiology showed an increased seizure threshold (8.2 ± 1.9 V) compared with their *top1*^{G0229} *eas/+ eas* controls (3.4 ± 0.2 V). Formally, *top1*^{G0229} in heteroallelic combination with *top1*^{JS} appears to be a recessive seizure suppressor of *eas*. Qualitatively similar results were observed with other mutations in heteroallelic combination with *top1*^{JS}: *top1*^{112} (34% BS suppression, 6.3 ± 2.5 V seizure threshold), *Df(1)ED7294* (68% BS suppression, 10.4 ± 2.7 V seizure threshold). In contrast, several *top1* lethal mutations show no evidence of seizure suppressor function: they were found to complement seizure suppression by *top1*^{JS} (Fig. 2*A*,*B*). For example, *top1*^{G0201} does not appear to be a seizure suppressor, as indicated by 0% suppression seen in flies of the genotype *top1*^{G0201} *eas/top1*^{JS} *eas* identical to their control genotype *top1*^{G0201} *eas/+ eas*. The two genotypes also show similar seizure thresholds: 3.5 ± 0.2 V for the experimental compared with 3.3 ± 0.1 V for the control. Qualitatively similar results were seen with *top1*^{G0134} in heteroallelic combination with *top1*^{JS} (5% BS suppression, 4.2 ± 1.2 V seizure threshold).

In some *top1*^{JS} *eas* flies, paralysis is completely eliminated; others become paralyzed but recover more quickly, a phenomenon previously seen in BS mutants treated with human anticonvulsant drugs (Reynolds et al., 2004; Tan et al., 2004; Song and Tanouye, 2006), as well as double-mutant combinations of BS mutants with their corresponding seizure-suppressor mutants (Song and Tanouye, 2006). Normally, *eas* mutants require 44 ± 11 s to recover from paralysis when flies are 2 to 3 d old; it only takes same-age *top1*^{JS} *eas* flies 27 ± 4 s to recover (Fig. 2*C*). Recovery time is another criterion to judge the seizure severity in BS mutants and double mutants of BS mutants with seizure-suppressor mutants.

Seizure suppression by *top1*^{JS} mutation is reverted by introducing wild-type *top1*

To further investigate whether the suppression by *top1*^{JS} is caused by reduced expression of *top1*, we crossed *top1*^{JS} *eas* double mutants to flies carrying a genomic duplication of the *top1* gene. The

top1 rescue construct used here is *P {top1 ± 10}*, which contains a 10 kb genomic *Spe*I–*Bam*HI fragment. This 10 kb genomic fragment includes the entire *top1* transcribed region, 1.3 kb of upstream and 0.5 kb of downstream sequences; it contains no complete gene other than *top1* (Lee et al., 1993) (http://flybase.bio.indiana.edu). We found that the addition of an extra copy of *top1+* product successfully restored the behavioral bang-sensitive phenotype of $top1^{JS}$ *eas* double mutants. The percentage of bang sensitivity increased from 36% in $top1^{JS}$ *eas* double-mutant flies to 100% in $top1^{JS}$ *eas; P {top1 ± 10}/+* flies. In electrophysiology experiments, the $top1^{JS}$ *eas* double mutants have seizure thresholds at 9.1 ± 1.1 V, and the addition of an extra copy of *top1+* decreased the seizure threshold of $top1^{JS}$ *eas* to 3.4 ± 0.2 V, which is very similar to the seizure threshold of the *eas* mutant (Fig. 3). The restoration of seizure sensitivity in both behavioral testing and electrophysiological experiments by adding *top1+* is another piece of evidence that $top1^{JS}$ reduces *top1* expression, leading to seizure suppression.

Precise excision of the P element in the double-mutant $top1^{JS}$ *eas* restores seizure susceptibility similar to the *eas* mutant

Excision experiments were performed by reintroducing the transposase Delta 2,3 into the $top1^{JS}$ *eas* double mutants. Among eight putative precise excision lines, one precisely excised line was verified according to genomic PCR and sequencing assays. Behavioral and electrophysiological experiments showed that this precise excision line restores the behavioral bang-sensitive phenotype to 100% and the seizure threshold to 3.3 ± 0.2 V (Fig. 3). The other seven putative precise excision lines turned out to contain small insertions varying from several base pairs to ~200 bp. Interestingly, despite these small insertions, flies showed bang-sensitive behavioral phenotypes and low seizure threshold, similar to the precise excision lines. Surprisingly, we did not find any imprecise excision lines that deleted *top1*. Our expectation was that *top1* deletions would be bang resistant (viable) or lethal; this would be somewhat similar to *Df(1)ED7294*, which deletes a region including the *top1* gene.

The $top1^{JS}$ mutation reveals seizure threshold similar to the wild type

When analyzed outside the BS background by electrophysiology, $top1^{JS}$ flies exhibit normal seizure thresholds like those of wild-type flies (Fig. 4*A*): the wild-type female fly has a seizure threshold of 41.5 ± 5.6V, whereas the $top1^{JS}$ mutant female has a seizure threshold of 36.7 ± 2.7V. In addition, the heteroallelic combinations $top1^{JS}/top1^{G0229}$, $top1^{JS}/top1^{112}$, $top1^{JS}/top1^{77}$, $top1^{JS}/top1^{G0134}$, and $top1^{JS}/top1^{G0201}$ have seizure thresholds similar to wild type (Fig. 4*A*). To further study whether alterations in individual neuron excitability lead to seizure suppression, GF thresholds were measured in the *top1* mutants. Results in Figure 4*B* showed that stimulus voltages required for activation of the GF in $top1^{JS}$ mutants and were indistinguishable from wild-type flies. This suggests that altered single neuron excitability is probably not an explanation for seizure suppression by the *top1* mutants.

All previously characterized seizure suppressors identified by reverse genetics exhibited elevated seizure thresholds that are significantly higher than those of wild type. Thus, the seizure-suppressor mutants $shakB^{2}$ (gap junction) and *para* (voltage-gated Na^+ α-subunit) exhibit seizure thresholds of 80.63 ± 8.71 and 65.0 ± 7.2 V at 300 ms HF stimuli, respectively; the seizure suppressor mutants mle^{napts} (double-strand RNA helicase), Sh^{KS133} (voltage-gated K^+ α-subunit), and $mei\text{-}P26^{EG}$ (RBCC-NHL protein) exhibit seizure thresholds of 72.2 ± 7.3, 83.8 ± 12.8, and 91.4 ± 5.1 V at 400 ms HS stimuli, respectively (Kuebler et al., 2001; Glasscock et al., 2005; Song and Tanouye, 2006). The ectopic neural expression of *esg* (*escargot*) (transcription factor of snail family) raised the seizure threshold to 55.5 ± 13.2 V (Hekmat-Scafe et al., 2005). The elevated seizure threshold was thought to relate reduced seizure susceptibility (Kuebler et al., 2001). The normal seizure threshold and GF threshold displayed in $top1^{JS}$ mutation may indicate a different mechanism of seizure suppression than other mutants above. A more likely possibility is that a positive feedback loop, which recruits multiple neurons simultaneously is disrupted so that the seizure would be suppressed or shortened. Another possibility is that suppression is a result of synaptic inhibition: the recruitment of inhibitory circuits, instead of excitatory (seizure) circuits, could account for the suppression and shortening of seizures we see near the suppression threshold.

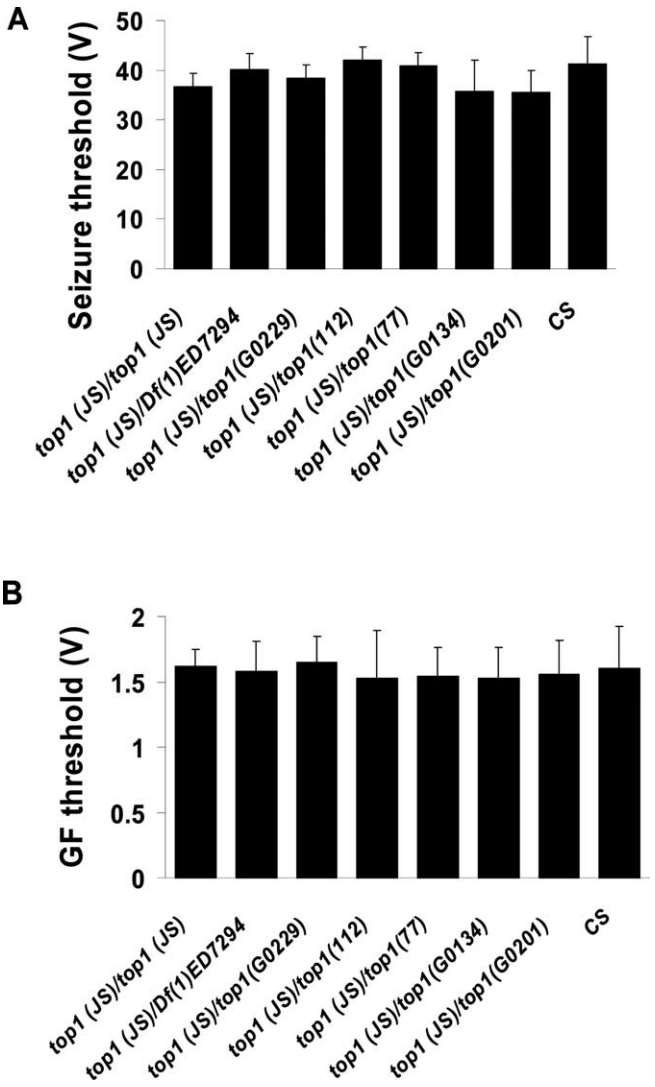

Figure 4. Seizure thresholds and GF thresholds of $top1^{JS}$ and its various heteroallelic combinations. ***A***, Seizure thresholds of $top1^{JS}$ mutation and its various heteroallelic combinations. ***B***, GF thresholds of $top1^{JS}$ and its various heteroallelic combinations. GF thresholds were measured by determining the minimum voltage required to elicit a stable, short-latency DLM response (~1.4 ms) after 0.5 ms test pulses delivered at 1 Hz. For each genotype, $n \geq 8$ flies tested. For both seizure threshold and GF threshold, results of different allelic combinations do not show significant difference compared with wild-type CS flies ($p > 0.05$).

The *top1^{JS}* mutant acts as a general seizure suppressor

Additional genetic experiments revealed that the *top1^{JS}* mutation interacts with other BS mutations, suggesting that it functions as a general seizure suppressor (Fig. 5). In general, *bss* is regarded as the most difficult BS mutant to suppress, followed by *eas*, and then *sda*. Mutants of *bss* have a seizure threshold at 3.2 ± 0.3 V, whereas *sda* flies have seizure thresholds ~6 V, higher than those of both *eas* and *bss*. As shown in Figure 5*A*, the bang-sensitive phenotype of *sda* was strongly suppressed by the *top1^{JS}* mutation, resulting in 63% suppression for *top1^{JS}; sda* double mutants. Control flies of both *sda* and *top1^{JS}/+; sda/sda* showed 100 and 95% bang sensitivity, respectively. In electrophysiology experiments, *top1^{JS}* elevated *sda* seizure threshold to 14.8 ± 4.9 V, much higher than that of the *sda* mutant itself (Fig. 5). The behavioral and electrophysiological data indicated that t*op1^{JS}* acts as a general seizure suppressor and does not interact solely with the *eas* mutation. To further investigate a role for *top1* in seizure suppression, we examined additional *top1* mutants in a *sda* background. As for *eas*, some, but not all, top1 alleles acted to suppress *sda* seizures (Fig. 5). Thus, *top1^{112}* acts to suppress *sda* seizures as indicated by the genotype: *top1^{JS}/top1^{112}; sda* that showed 61% suppression of the behavioral paralytic phenotype compared with their control genotypes. In addition, electrophysiology showed an increased seizure threshold (14.2 ± 1.8 V), compared with their control genotypes (~6 V). Finally, *top1^{112}* in heteroallelic combination with *top1^{JS}* appears to be a recessive seizure suppressor of *sda*. Several *top1* lethal mutations show no evidence of seizure suppression function: they were found to complement seizure suppression by *top1^{JS}*. That is, *top1^{G0229}*, *top1^{G0201}*, and *top1^{G0134}* all do not suppress *sda* seizures in heteroallelic combination with *top1^{JS}*. For *top1^{G0201}* and *top1^{G0134}*, these findings are similar to *eas*; these *top1* mutations did not act as seizure suppressors. In contrast, the findings with *top1^{G0229}* are especially interesting because it is a potent suppressor of *eas* seizures (Fig. 2) but not *sda* seizures.

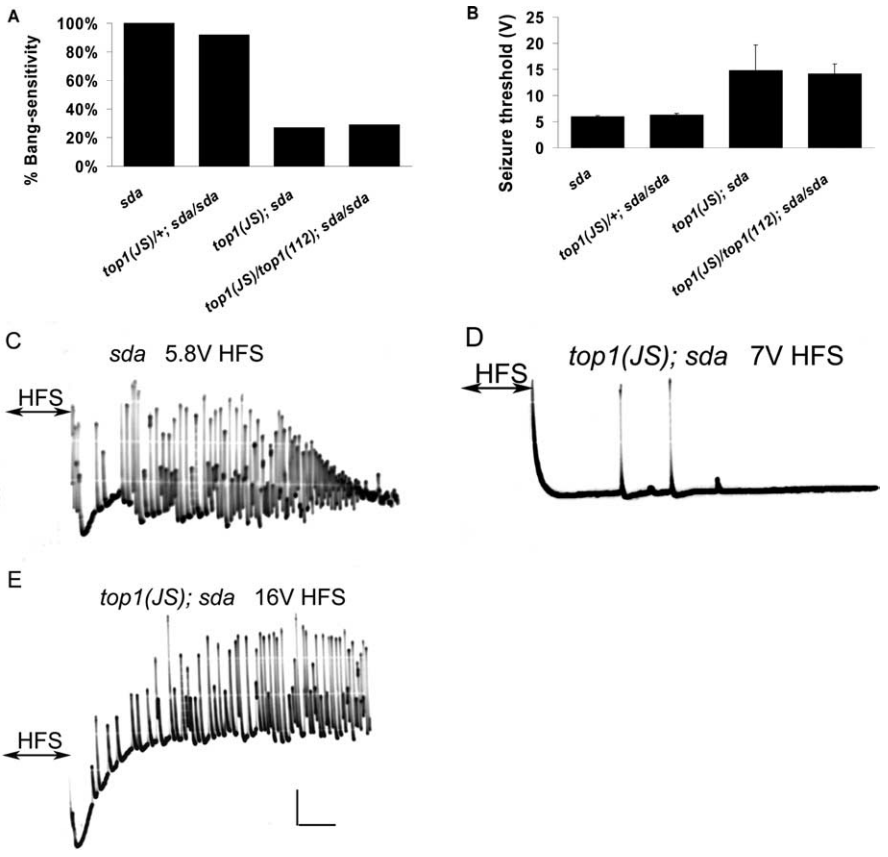

Figure 5. The *top1* mutation suppresses seizures in *sda* flies. ***A***, Behavioral suppression of seizures in *sda* flies by *top1^{JS}* and *top1^{JS}/top1^{112}* heteroallelic combination. The *top1^{JS}* mutation and *top1^{JS}/top1^{112}* heteroallelic combination reduce bang-sensitive paralysis in *sda* flies to 27 and 29%, respectively ($p < 0.01$). Suppression of the *sda* BS behavior by *top1^{JS}* is semidominant because *top1^{JS}* heterozygotes are 92% bang sensitive. ***B***, Electrophysiological suppression of seizures in *sda* flies by *top1^{JS}* and *top1^{JS}/top1^{112}* heteroallelic combination. Seizure thresholds are elevated in flies of genotypes *top1^{JS}; sda* and *top1^{JS}/top1^{112}; sda* ($p < 0.01$). All *sda* genotypes were examined in females. ***C***, A representative seizure in an *sda* fly recorded from the DLM after a high-frequency brain stimulus (HFS) of 5.8 V. ***D***, failure to elicit seizure in a *top1^{JS}* fly after a 7 V HFS. The trace shown here is absent of seizure activity, and only a few DLM responses can be seen after administration of a 7 V HFS. ***E***, A seizure is elicited in a *top1^{JS}; sda* double mutant after a 16 V HFS. Calibration: 10 mV, 200 ms. For each genotype, $n \geq 80$ flies tested for behavior; $n \geq 8$ flies tested for electrophysiology.

The bang-sensitive behavioral phenotype of *bss* was not suppressed by *top1^{JS}*. Thus, *top1^{JS} bss* double mutants showed 100% bang sensitivity. However, *top1^{JS} bss* does appear to change some *bss* phenotypes, indicating that it may act to reduce the severity of *bss* seizures. This is most apparent in a reduction of paralytic behavioral recovery time. Thus, recovery time of the *bss* mutant is 120 s (1–2 d); recovery time is shortened to 77 s in the *bss* double mutants (Fig. 6). It has been shown previously that a reduction in recovery time, mainly a reduction in tonic–clonic spontaneous activity in *bss* mutants, is the first indication of seizure suppression (Kuebler et al., 2001; Song and Tanouye, 2006). In addition, the *top1^{JS}* mutation suppresses the behavioral bang sensitivity of *bss*/+ heterozygotes. The *bss* mutant behaves as a dominant mutation in behavior, that is, *bss*/+ heterozygotes show 100% bang sensitivity. In contrast, *top1^{JS} bss/top1^{JS}* + flies only show 12% bang sensitivity (Fig. 6), suggesting suppression of the BS phenotype of *bss*/+ by the *top1^{JS}* mutation.

The *top1^{JS}* mutant has a reduced topoisomerase I mRNA transcription level in the CNS

Reverse-transcription PCR showed that *top1* transcripts were reduced in the heads of *top1^{JS}* mutants. Wild-type flies displayed a strong expression level of *top1* transcripts, whereas the *top1^{JS}* mutant displayed a weak expression level of *top1* transcripts (Fig. 7). There are three *top1* transcripts in *Drosophila* referred to as top1-RA (5325 nt), top1-RB (2379 nt), and top1-RC (4940 nt). Because of the presence of large overlapping regions among the three transcripts, this assay could not differentiate the transcription level of individual mRNA transcript. Shown in Figure 7 is the mRNA transcription level of *top1*: the mutant transcription level is ~12.5-fold less than wild type. This transcription profile indicated that *top1^{JS}* is a partial loss-of-function mutation caused by the disruption of 5′ UTR, and seizure suppression by the *top1^{JS}* mutation is caused by reduced level of *top1* transcripts.

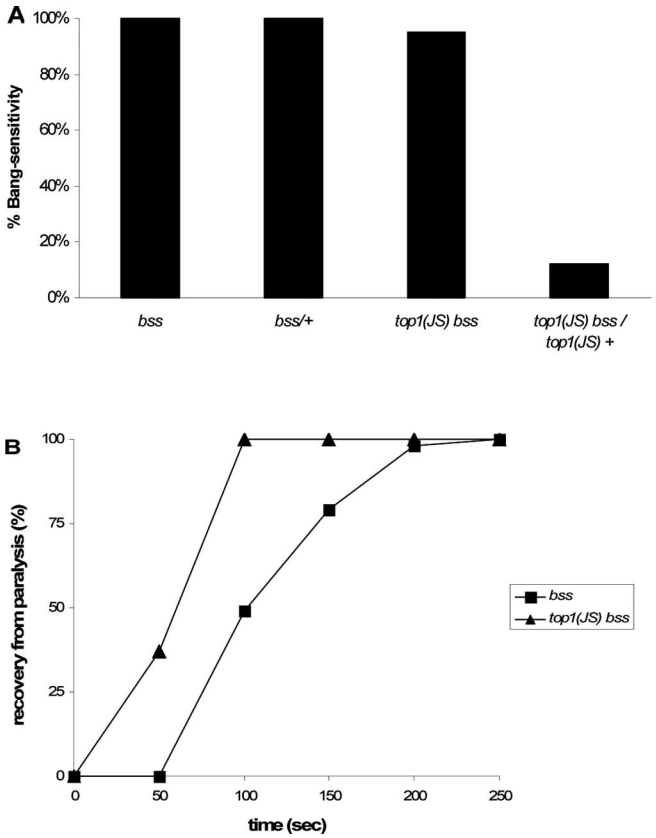

Figure 6. Seizure suppression in *bss* flies. ***A***, Behavioral bang sensitivity is reduced in *bss*/+ heterozygous flies in the presence of $top1^{JS}$. Note that *bss*/+ flies show 100% bang sensitivity; however, when it is placed in a $top1^{JS}$ homozygous background, behavioral bang sensitivity is reduced to 12% ($p < 0.01$). ***B***, The recovery time is shortened in double-mutant $top1^{JS}$ *bss* flies. The *bss* mutant has an average paralysis time of 120 s, whereas the double-mutant $top1^{JS}$ *bss* shorten the paralysis time to 77 s. Shown is the percentage of flies recovered with time and a cumulative measure that includes the initial behavior seizure, the paralysis period, and the recovery seizure, which are not indicated separately. The number of flies standing at intervals after the shock was recorded until the entire population had recovered. For behavioral bang-sensitivity test, $n \geq 50$ flies tested; for the recovery time, $n \geq 30$ flies recorded.

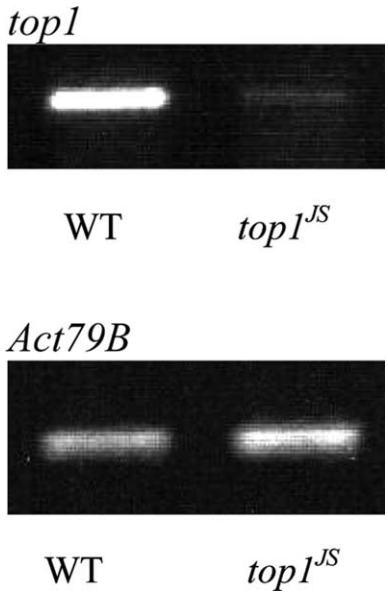

Figure 7. RT-PCR reveals a reduction in the *top1* mRNA transcription level. Shown are the results of an RT-PCR analysis of RNA prepared from the heads of wild-type (WT) flies and $top1^{JS}$ mutant flies. Primers used in this assay amplified part of the first exon of the *top1* gene. The internal control used in this assay is fly actin gene *CG7478-PA* (*Act79B*). The heads of $top1^{JS}$ mutants display a significant reduction (~12.5-fold) in the level of *top1* transcripts. Calibration: 10 mV, 200 ms.

Table 1. Effects of camptothecin on the recovery time of various BS mutants

	Recovery time (s)				
Genotype	Control (−CPT)	*n*	Test (+CPT)	*n*	*p* value
bss	118 ± 43	80	55 ± 9	65	<0.01
eas	44 ± 11	80	30 ± 5	62	<0.01
sda	25 ± 6	80	21 ± 3	72	<0.05

Adult flies were fed on the CPT-containing medium, and their progeny were tested at 1–3 d after eclosion for the recovery time. Control flies were fed CPT-free medium. Results are statistically significant for all three mutants ($p < 0.05$).

Camptothecin reduces recovery time in BS mutants

We examined effects of the topo I inhibitor camptothecin (CPT) in three BS mutants: *eas*, *sda*, and *bss*. These mutants were raised in various concentrations of the drug, and their bang sensitivity was examined. In our studies, we did not see the elimination of the BS phenotype in any of our mutants treated with CPT. However, we did observe a reduction in the paralytic recovery time of all three BS mutants (Table 1). Treatment of *bss* with 1 μM CPT shortened the recovery time to 55 ± 9 s ($n = 65$) compared with the untreated (118 ± 43 s; $n = 80$) recovery time. There was also a reduction in the *eas* recovery time, albeit, more modest than seen for *bss*. Treatment of *eas* with 4 μM CPT shortened the recovery time to 30 ± 5 s ($n = 62$) compared with the untreated (44 ± 11 s; $n = 80$) recovery time. We also observed a small but statistically significant reduction in the *sda* recovery time. Treatment of *sda* with 1 μM CPT shortened the recovery time to 21 ± 3 s ($n = 72$) compared with the untreated (25 ± 6 s; $n = 80$) recovery time. These observations are generally consistent with those described previously using anti-epileptic agents fed to BS flies. Paralytic recovery time is reduced by phenytoin, gabapentin, KBr, and carbenoxolone: anti-epileptic drugs with different targets (Reynolds et al., 2004; Tan et al., 2004; Song and Tanouye, 2006). Some anti-epileptic drugs (carbamageprine, ethosuximide, and vigabactrin) have no effect on BS flies (Reynolds et al., 2004). Anti-epileptic drugs generally do not eliminate the BS phenotype of mutants (Reynolds et al., 2004).

The $top1^{JS}$ mutant has increased cell death

Seizure suppression by the $top1^{JS}$ mutant might be caused by neuronal cell death. This is consistent with increased neuronal cell death observed with treatment of the topoisomerase I inhibitor camptothecin (Morris et al., 1996). TUNEL assay showed increased apoptosis in $top1^{JS}$ mutant brain compared with that observed in wild-type flies (Fig. 8*A*,*B*). In addition, gel electrophoresis of $top1^{JS}$ mutant and $top1^{JS}$ *eas* double-mutant DNA showed considerable fragmentation compared with wild-type (Fig. 8*C*). The indication is that the $top1^{JS}$ mutation causes considerable cell death, much of it in the nervous system.

Overexpression of DIAP1 in the nervous system restores seizure sensitivity in $top1^{JS}$ *eas*

DIAP1, which is encoded by the *th* (*thread*) gene, is an antiapoptotic protein. *DIAP1* contains two BIR (baculoviral inhibitor of apoptosis repeats) domains that are required for its antiapoptotic function. The GAL4/UAS expression system was used: the embryonic lethal abnormal vision (ELAV)–GAL4 construct leads to neuronal expression of GAL4 transcriptional activator, which drives expression of UAS–DIAP1 in all neurons. Overexpression

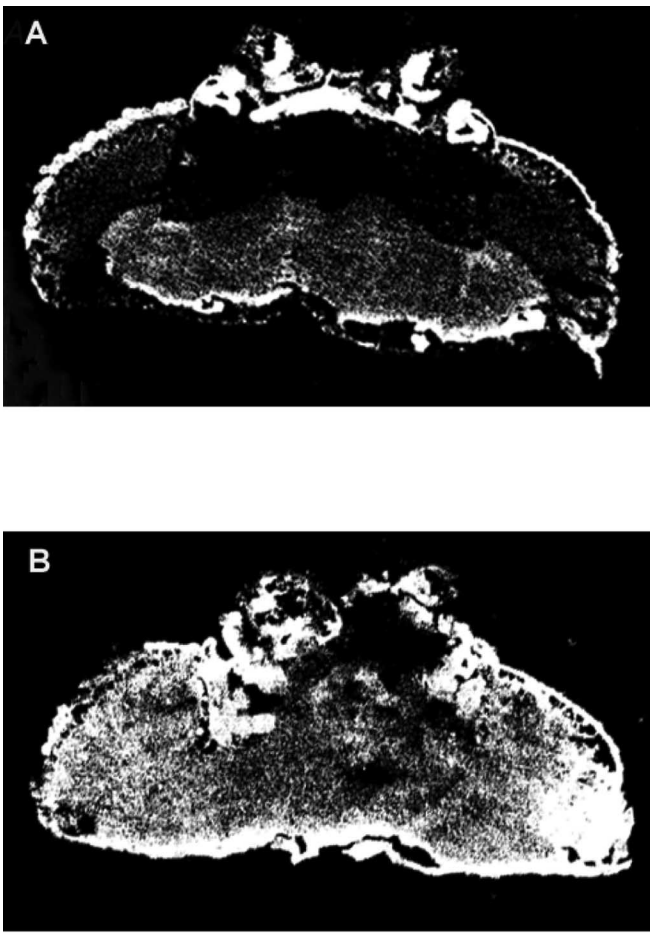

Figure 8. The *top1*^{JS} mutation causes apoptosis. ***A***, ***B***, TUNEL assay. Representative sections from adult brain of wild-type (***A***) and *top1*^{JS} mutant (***B***) flies. White indicates TUNEL signal. Weak diffuse TUNEL is observed in wild type. In mutant, TUNEL signal is more widespread and intense. ***C***, DNA analysis by 1.5% agarose gel electrophoresis of genomic DNA. Lane 1, DNA ladder, 1 kb (Fermentas, Hanover, MD). Lane 2, Genomic DNA from wild-type flies. Lane 3, DNA from *top1*^{JS} *eas* double-mutant flies. Lane 4, DNA from *top1*^{JS} mutant flies. Fragmentation is observed in mutant DNA.

of DIAP1 in the nervous system should suppress neuronal apoptosis, leading to the rescue of any neuronal apoptosis phenotypes caused by the *top1*^{JS} mutation. This rescue could be reflected by restored seizure sensitivity in *top1*^{JS} *eas* double mutants. Indeed, flies with the genotype *top1*^{JS} *eas; P(ELAV-GAL4)/P(UAS*-DIAP1) revealed substantial seizure sensitivity (Fig. 9): the behavioral bang sensitivity was restored to 100%, similar to the *eas* single mutant (Fig. 9*A*); the electrophysiological seizure threshold was decreased to 4.9 ± 1.1 V, significantly lower than the 9.8 ± 1.3 V in *top1*^{JS} *eas* double mutants (Fig. 9*B–D*). This result suggests that the seizure suppression caused by the *top1*^{JS} mutant is a result of increased neural apoptosis, pointing to a novel function for topo I in construction and/or maintenance of a circuit required for seizure propagation *in vivo*.

Discussion

Discovery of a novel seizure-suppressor mutation: *top1*^{JS}

The identification of *top1* as a seizure suppressor is surprising because the biological functions with which it has been associated are not obviously electrical excitability functions. The *top1* gene appears to play an important role in the nervous system as a key regulator of seizure susceptibility. The *top1*^{JS} mutation shows the ability to suppress seizures in multiple BS strains, including *eas*, *sda*, and *bss* mutants. These BS mutants are well characterized on a behavioral and electrophysiological level, and their seizure activity shows numerous similarities with seizure activity in humans, making them a useful tool for identifying new seizure suppressors (Benzer, 1971; Kuebler and Tanouye, 2000). Two of these BS mutants examined (*eas* and *sda*) encode very different products: *eas* encodes ethanolamine kinase involved in synthesis of the phosphatidyl ethanolamine in neuronal membrane, and *sda* encodes an aminopeptidase N (Pavlidis et al., 1994; Zhang et al., 2002). The gene product of *bss* is still unknown but thought to encode another very different product (M. Tanouye, unpublished results). Therefore, these three BS mutations may increase seizure susceptibility of flies by different mechanisms. The *top1*^{JS} mutation can suppress seizures in *eas* and *sda* flies to a similar extent, with the suppression slightly better in *top1*^{JS}; *sda* double mutants than in *top1*^{JS} *eas* double mutants, whereas the suppression of behavioral bang sensitivity in *bss* flies is not very efficient. However, the recovery time after paralysis is drastically shortened, and the tonic–clonic spontaneous seizures during paralysis are drastically reduced in double-mutant *top1*^{JS} *bss* flies. In addition, *top1*^{JS} suppresses seizures in *bss*/+ heterozygotes to 12% behaviorally (100% in *bss*/+ flies). This is consistent with previous observations on general seizure suppressors that *bss* is the strongest of the three mutations, *eas* is the intermediate, and *sda* is the weakest (in terms of reduction of seizure thresholds and the ease with which it can be suppressed by secondary mutations that reduce nervous system excitability).

The partial loss-of-function *top1*^{JS} mutations that act to suppress seizures cause no other obvious phenotypes whether in a wild-type or a seizure-sensitive mutant background. Thus, *top1*^{JS} mutant flies show no obvious nervous system excitability defects: they are not temperature paralytic (hypoexcitability), and they do not exhibit leg-shaking under ether anesthesia (hyperexcitability). In addition, the mutant flies show no notable defects in other specific behaviors: they eat, jump, fly, groom, court, and mate normally. The *top1* alleles characterized previously all exhibit homozygous lethal phenotype, suggesting an essential role of the *top1* gene in *Drosophila* development. The *top1*^{JS} mutation is the first homozygous viable *Drosophila* partial loss-of-function *top1* mutation identified thus far. Molecular experiments verified that

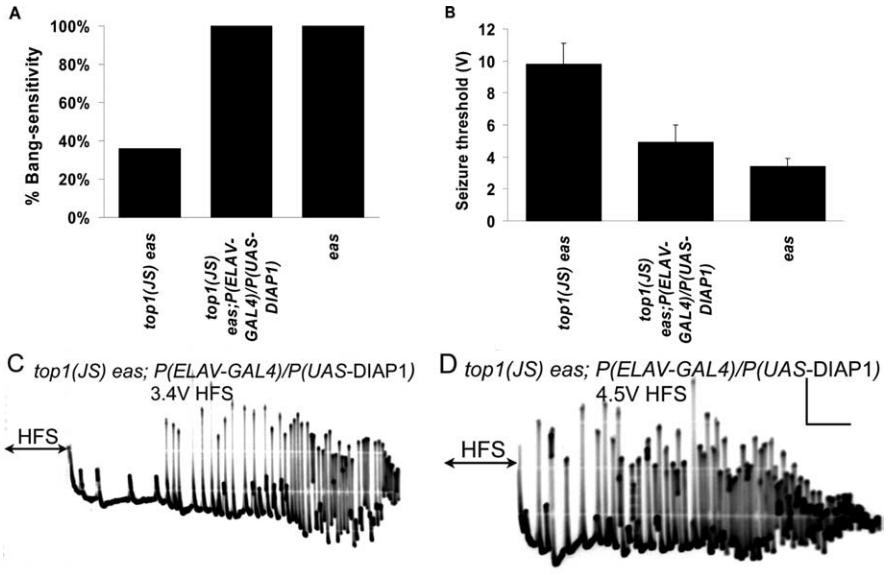

Figure 9. Overexpression of DIAP1 in the nervous system restores seizure sensitivity of $top1^{JS}$ *eas* double mutants. ***A***, Behavioral bang sensitivity is restored in $top1^{JS}$ *eas; P(ELAV-GAL4)/P(UAS*-DIAP1) flies ($p < 0.01$, compared with $top1^{JS}$ *eas*). ***B***, The seizure threshold is decreased in $top1^{JS}$ *eas; P(ELAV-GAL4)/P(UAS*-DIAP1) flies compared with $top1^{JS}$ *eas* ($p < 0.01$), suggesting increased seizure sensitivity. ***C***, A seizure is triggered in a $top1^{JS}$ *eas; P(ELAV-GAL4)/P(UAS*-DIAP1) fly at a 3.4 V HFS. ***D***, A seizure is triggered in a $top1^{JS}$ *eas; P(ELAV-GAL4)/P(UAS*-DIAP1) fly at a 4.5 V HFS. Calibration: 10 mV, 200 ms. For each genotype, $n \geq 30$ flies tested for behavior; $n \geq 6$ flies tested for electrophysiology.

the mutation has a reduced mRNA transcription level compared with wild-type flies.

There appears to be two salient phenotypes associated with *top1* mutations: (1) recessive lethal, suggesting a vital function, and (2) a recessive seizure suppression function. A parsimonious model for the results from different *top1* alleles might have $top1^{JS}$ as the weakest allele because it is the only viable one. Furthermore, the lethal alleles would be expected to have stronger phenotypes indicative of greater loss-of-function. This does not appear to be the case: *top1* gene function seems more complicated based on several observations involving allelic specificity. Although viable, $top1^{JS}$ appears to be the strongest seizure suppressor: greater seizure suppression is seen for $top1^{JS}$ homozygotes than for any of the heteroallelic combinations. Surprisingly, in heteroallelic combinations, some *top1* lethal alleles ($top1^{G0134}$, $top1^{G0201}$) complement suppression of *eas* seizures. Another interesting observation from examination of different *top1* alleles comes from results with $top1^{G0229}$. In heteroallelic combination with $top1^{JS}$, this allele suppresses *eas* seizures, but not *sda* seizures. The surprise is that several previous studies have shown that *sda* is generally easier to suppress than *eas*. The explanation for these *top1* allelic complexities is not entirely clear at present. The P-element insertion sites differ for the different *top1* alleles, and part of the explanation may come from differential exon usage in different *top1* isoforms.

Possible mechanism of seizure suppression by $top1^{JS}$

The mechanism by which topo I influences seizure susceptibility is not clear. Mouse brain shows substantial levels of topo I activity varying among different brain regions (Plaschkes et al., 2005). Immunohistochemistry shows that the highest topo I levels are observed in inhibitory neurons. *In vitro* studies show that topo I disruption leads to neuronal cell death, evidenced by accompanying DNA fragmentation, chromatin condensation, cell blebbing, cytoplasmic shrinkage, fragmentation of neurites, and other characteristics of apoptotic cell death (Morris and Geller, 1996). RNA and protein inhibitors prevent apoptosis attributable to topo I disruption. This indicates that topo I-dependent apoptosis requires active transcription and translation of downstream proteins involved in apoptosis and other topo I-interacting proteins. One possible mechanism is adapted from a recently proposed model of topo I action in DNA relaxation and damage control (Leppard and Champoux, 2005). Transcription in neurons generates supercoiled DNA that must be continuously relaxed to sustain high levels of RNA synthesis. Binding of topo I and DNA forms the cleavable complex leading to relaxation. A reduced level of topo I attributable to mutation or CPT treatment leads to partial inhibition of transcription. A series of events including relocalization of topo I from the nucleolus to the nucleoplasm leads to downstream events: the synthesis of proteins involved in the apoptosis. Electrophysiological evidence provided in this study is consistent with the above explanation. In addition, apoptosis analysis showed that $top1^{JS}$ caused considerable amounts of cell death. Furthermore, overexpression of *DIAP1* in the nervous system resulted in the restoration of seizure sensitivity in $top1^{JS}$ *eas* double mutants, suggesting increased apoptosis in the $top1^{JS}$ mutant is the cause of seizure suppression.

The $top1^{JS}$ mutation has a normal seizure threshold compared with wild-type flies when analyzed outside a BS background. A model that accounts for several major features of *Drosophila* seizure susceptibility and its modification by genetic mutations was proposed (Kuebler et al., 2001). This model proposed that the number of input neurons makes a difference in seizure thresholds among wild-type, BS mutant, seizure suppressor mutant, and double mutants of BS mutant with suppressor mutant. In a normal wild-type fly, stimulation of two input neurons (each input neuron represents a population of neurons with similar properties or an extensive neural circuit) with a high-frequency electrical stimulus triggers a seizure, and synaptic potentials from these two neurons summate in the Sei (seizure) neuron temporally and spatially. Seizure is triggered through the seizure output neural circuit once the threshold is reached. In a BS mutant, it is easier to trigger a seizure, and stimulation of a single neuron is sufficient. A low-voltage high-frequency stimulus is sufficient to trigger a seizure. In a seizure suppressor mutant, it is difficult to trigger a seizure and necessary to trigger three input neurons. Therefore, in this case, a high-voltage high-frequency stimulus is required. In a double mutant, the seizure threshold is elevated and higher than a BS mutant, so only two neurons are stimulated to trigger a seizure, and they are summated temporally and spatially. Based on this model, neuronal cell death caused by the $top1^{JS}$ mutation decreases the number of neurons being stimulated during the high-frequency stimulation. Therefore, a normal seizure threshold was seen in the $top1^{JS}$ mutant. The neuronal cell death proposal can also explain the discrepancy seen in electrophysiology and behavior. The $top1^{JS}$ mutation can suppress *bss*/+ flies to 12% bang sensitivity behaviorally (100% bang sensitivity in *bss*/+

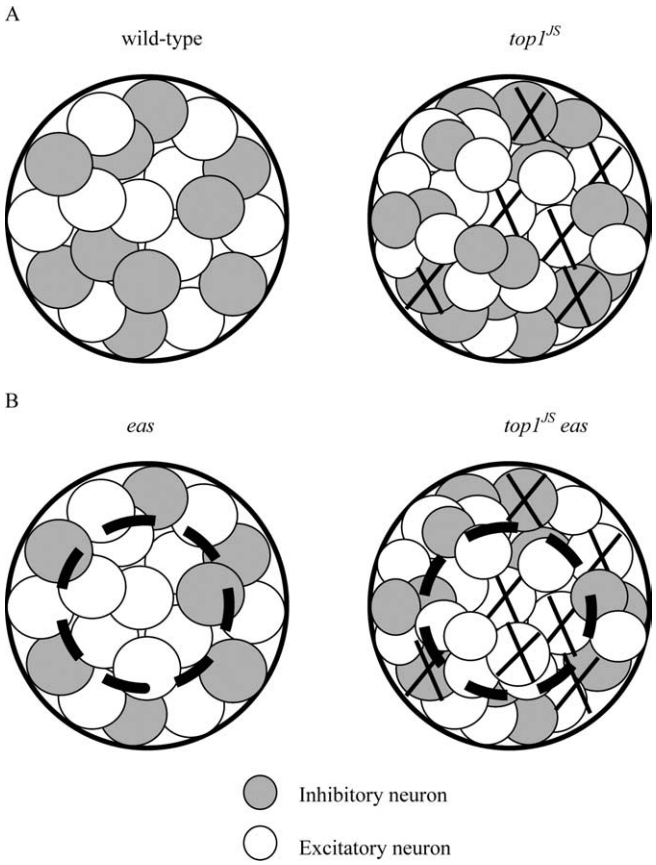

Figure 10. Sei neuron pools in different genetic backgrounds. ***A***, The Sei neuron pool in wild type and the *top1*^{JS} mutant. Excitatory and inhibitory neurons are balanced in the Sei neuron pool, although the *top1*^{JS} Sei neuron pool contains more neurons than the wild-type because of its seizure-resistant property. ***B***, The Sei neuron pool in the *eas* mutant and *top1*^{JS} *eas* double mutant. Excitatory neurons are more prevalent than inhibitory neurons in the Sei neuron pool because of the seizure-sensitive property of *eas* mutant background. The dot-circled area represents the neurons involved in seizure generation, because lower seizure thresholds are required in *eas* and *top1*^{JS} *eas* than the wild type and *top1*^{JS}.

flies); however, the seizure threshold remains unchanged compared with that of *bss*/+ flies.

The above model seems to present us with a paradox. Seizure threshold of the *top1*^{JS} mutant is similar to the wild type because of increased neuronal apoptosis; therefore, seizure threshold of double-mutant *top1*^{JS} *eas* should be lower than the *eas* mutant itself also because of increased neuronal apoptosis. However, in contrast, seizure threshold of the *top1*^{JS} *eas* double mutant is actually higher than the *eas* single mutant, as seen in the previous electrophysiological assay. One possible explanation for this paradox could be that different genetic backgrounds generate different ratios of excitatory neurons to inhibitory neurons in the Sei neuron pool (Fig. 10). In the wild-type and *top1*^{JS} mutant flies, excitatory and inhibitory neurons are balanced in the Sei neuron pool, although the *top1*^{JS} Sei neuron pool contains more neurons than the wild-type because of its seizure-resistant property. When neuronal apoptosis occurs, excitatory and inhibitory neurons are killed at the same level; therefore, the total number of neurons is similar in the wild-type and the *top1*^{JS} mutant, leading to similarities in seizure threshold (Fig. 10*A*). In the double-mutant *top1*^{JS} *eas* and *eas* mutant flies, excitatory neurons are more prevalent than inhibitory neurons in the Sei neuron pool because of the seizure-sensitive property of *eas* mutant background. Therefore, when neuronal apoptosis occurs, more excitatory neurons are killed than inhibitory neurons in the double-mutant *top1*^{JS} *eas*. In this case, to reach the seizure threshold, more (excitatory/inhibitory) neurons are required to be recruited to trigger a seizure, which results in the increased threshold in *top1*^{JS} compared with the *eas* mutant (Fig. 10*B*).

Practical implications from genetic and CPT-feeding analyses

Epilepsy is the most common chronic neurological disorder, affecting ~2 million people in the United States population (McNamara, 1999; Shneker and Fountain, 2003). However, only approximately two-thirds of patients experience symptomatic relief with antiepileptic drugs currently on the market, and many of these responders experience periodic breakthrough seizures and toxic nervous system side effects. The present work is an example of how *Drosophila* genetics can lead to identification of new genes and new drug targets relevant to human disease. The identification of topo I as a seizure suppressor is surprising because DNA topoisomerases have not previously been associated with seizure or seizure control. For *top1*^{JS} mutants, there are no apparent defects in nervous system functions or other specific behaviors implying that limited disruption of topo I can be well tolerated. However, *top1* is an essential gene and severe *top1* mutations are lethal, implying that *top1* function must be adjusted to an ideal range: one that suppresses seizures but is not otherwise deleterious. CPT acts as an inhibitor of topo I in mammals. CPTs have been described as one of the most promising anticancer drugs of the 21st century. Irinotecan (CPT-11) and topotecan are water-soluble derivatives approved by the Food and Drug Administration for treatment of colorectal and ovarian cancer. CPT has not previously been associated with the treatment of epilepsy, but potential application in the clinic is suggested by this work. CPT did not affect BS paralysis in flies but did affect recovery time. Although not as impressive as *top1*^{JS} mutation in terms of seizure suppression, CPT treatment does not cause detectable neuronal apoptosis in brain sections of viable long-term feeding progeny (data not shown). The assumption is that CPT treatment may have weaker effects than the genetic mutation, which is consistent with the weaker behavioral seizure suppression by CPT. Moreover, CPT results are comparable with others observed with known human anti-epileptic drugs. A preliminary comparison of feeding experiments suggests that CPT may be a less effective anti-epileptic agent compared with phenytoin, but may be better than valproate, KBr, and carbenoxolone (Kuebler and Tanouye, 2002; Reynolds et al., 2004; Tan et al., 2004; Song and Tanouye, 2006).

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