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The Therapeutic Effect of Stiripentol In *Gabrg2^{+Q390X}* Mice Associated With Epileptic Encephalopathy

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Abstract

Anti-seizure drugs (ASDs) are widely used and known to increase inhibitory tone on neuro-circuits and reduce aberrant synchronous firing in epilepsy. Some ASDs act as agonist at the GABA_A receptor. Stiripentol, known to increase GABA_A receptor activity as well as the metabolites of GABA_A receptor agonists, is often used in the treatment of an epileptic encephalopathy, Dravet syndrome (DS), which is caused by mutations mainly in *SCN1A* and in other genes such as *GABRG2*. We have recently generated a *Gabrg2^{+Q390X}* knockin mouse model associated with DS in humans. The objective of the study was to explore the effects of stiripentol in DS with GABA_A receptor functional deficiency because of the etiology heterogeneity in DS. Monotherapy (stiripentol or Diazepam) and polytherapy (stiripentol and diazepam) treatments were tested in *Gabrg2^{+Q390X}* mice challenged with pentylenetetrazol (PTZ) seizure induction in conjunction with video-monitoring synchronized electroencephalogram (EEG) recordings. A combination of stiripentol and diazepam greatly reduced seizure-related events in *Gabrg2^{+Q390X}* mice following PTZ administration and increased survival. However, the treatment of stiripentol alone was mostly ineffective in alleviating seizure-related events except that it reduced mortality in PTZ challenged *Gabrg2^{+Q390X}* mice. The study suggests that stiripentol could be only used as add-on therapy for DS with GABA_A receptor functional deficiency, which is consistent with the most established clinical application of stiripentol. The study highlights the importance of mechanism-based precision treatment for DS considering the highly heterogeneous nature of etiology in DS and the fact that mutations in different genes give rise to the same clinical phenotype.

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DISCLOSURE

None of the authors have any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Keywords

stiripentol; Dravet syndrome; electroencephalogram; epilepsy; *GABRG2(Q390X)* mutation; benzodiazepine

INTRODUCTION

Epilepsy is a disorder characterized by atypical concurrent firing of neural circuits. Although many incidences of epileptic disorders are the result of a complex summation of genetic elements, single mutations in genes coding for the GABA_A receptor have been found to result in epileptic syndromes. For example, genetic mutations in *GABRG2* encoding $\gamma 2$ subunit of the GABA_A receptor have been associated with various epilepsy syndromes ((Harkin et al., 2002; Kang et al., 2009). Of the mutations in *GABRG2*, our focus is on a particular nonsense mutation *GABRG2(Q390X)* due to its association with more severe forms of epilepsy, Dravet syndrome (DS) — an epileptic encephalopathy that affects about one in 40,000 children under seven years old (Hurst, 1990). The severe symptoms associated with the *GABRG2(Q390X)* mutation are likely due to the fact that the truncated protein localizes to the endoplasmic reticulum and results in lowered gamma-aminobutyric acid (GABA)-evoked currents, exhibits a dominant-negative effect through the disruption of the normal functioning of wild-type subunits, and dimerizes to form high molecular mass protein complexes, which may cause neuronal dysfunction and contribute to neuronal death (Kang et al., 2015).

Treatments for epilepsy often focus upon anti-seizure drugs (ASDs). Many ASDs increase inhibitory tone to reduce aberrant synchronous neuronal firing. Some ASDs, such as benzodiazepines, act as positive allosteric modulators on GABA_A receptors. However, the treatment of epilepsy stemming from a *GABRG2* mutation is often not very effective with ASDs such as benzodiazepines alone — given the importance of the proper functioning of the $\gamma 2$ subunit of the GABA_A receptor (Pritchett et al., 1989). With this known, an alternative approach is to use ASDs conjointly. For example, stiripentol is an ASD that is typically co-administered with another anti-convulsant compound as an add-on therapy. It is mainly used because of its metabolic enzyme antagonistic properties although it can also potentiate GABA_A receptors. In particular, stiripentol and benzodiazepines act independently at GABA_A receptors, and implementing the polytherapy treatment appears to increase the maximum effectiveness compared to either type of drug alone (Fisher, 2011). For the current study, we examined the efficacy of stiripentol alone, diazepam alone (a benzodiazepine), and the co-administration of stiripentol and diazepam in a *Gabrg2^{+/Q390X}* knockin mouse model via electroencephalogram (EEG) assessment to provide an objective measure of seizure-related events and the effect of stiripentol in DS associated with *GABRG2* mutations.

METHODS

Mice

Gabrg2^{+/Q390X} heterozygous (het) knockin mice were characterized and used as previously studied (Kang et al., 2015), and their wildtype (wt) littermates were used as controls. We used *Gabrg2^{+/Q390X}* mice because it represents patient condition. Mice used in the study were bred into C57Bl/6J mice for at least 8 generations and were between 2–4 months old. All procedures were performed in accordance with policies and guidelines set forth by the Vanderbilt University Institutional Animal Care and Use Committee.

EEG recordings and Drug administration

EEG recordings were conducted based on our standard lab protocol (Kang et al., 2015; Warner et al., 2016). Stiripentol was kindly provided by Biocodex (Beauvais, France, 150 mg/kg). Stiripentol was initially dissolved in a 1:10 solution of dimethyl sulfoxide (DMSO) and 0.9 percent saline, then further diluted to a 1:100 concentration of corn oil before administered in animals. The volume injected into each mouse was about 200 μ l. Diazepam (0.3mg/kg) and pentylenetetrazol (50mg/kg) were obtained from Sigma-Aldrich (St. Louis, MO). All drugs were administered via intraperitoneal injection (IP). Stiripentol was administered 1 hr (Auvin et al., 2013) while Diazepam was administered 30 min (Warner et al., 2016) respectively before pentylenetetrazol treatment. Mice were routinely recorded for at least 24 hrs for baseline analysis before drug administration and at least 2 hrs after drug administration. For baseline recordings, the first 10 min for each hour were analyzed for 24 hrs while the first 15 min for each hour of the recordings after drug administration were analyzed. In the group with multiple drug treatment, the analysis started after the injection of the last drug. Both behavioral and electrographic seizure activity were monitored.

Statistical analysis

All data was analyzed with GraphPad Prism 5 software. While both male and female mice were used in the study, there were no sex-dependent effects (data not shown), so male and female mice were pooled together in all subsequent analyses. Independent-samples t tests were used for comparisons between genotypes and One sample t test was used when appropriate. A two-way analysis of variance (ANOVA) was used for the comparisons involved two factors and the post hoc test Bonferroni posttest was used for multiple comparisons. All analyses used an alpha level of 0.05 to determine statistical significance. Data were presented as Mean \pm SE.

RESULTS

Stiripentol alone increased myoclonic seizures but combination of stiripentol and diazepam reduced seizures in heterozygous *Gabrg2^{+/Q390X}* mice

A previous study indicated that *Gabrg2^{+/Q390X}* mice had increased seizure activities such as increased spontaneous myoclonic jerks (MJ) or myoclonic seizures, interictal discharges and generalized tonic clonic seizures (GTCS), so we wanted to determine the effect of stiripentol on seizure phenotypes in *Gabrg2^{+/Q390X}* mice. We evaluated the frequency of myoclonic

recordings without any injection (0.65 ± 0.03 for wt and 21.2 ± 3.5 for het), the SWDs were increased in the *Gabrg2^{+/Q390X}* mice treated with stiripentol (3.1 ± 1.3 for wt and 72.4 ± 8.6 for het, $P < 0.001$). Genotype and treatment were taken as independent variables. STP increased SWDs in both wildtype and heterozygous mice ($P < 0.001$ for interaction $F(1,43) = 39.93$; genotype $F(1,43) = 136.8$ and for drug treatment $F(1,43) = 50.22$); $N = 15$ for wt control, $N = 8$ for wt STP; $N = 18$ for het control and 8 for het STP, Figure 1C). Next, the effect of stiripentol, diazepam, or the combination of stiripentol and diazepam was determined in conjunction with PTZ challenge. There are more SWDs observed in the *Gabrg2^{+/Q390X}* mice in the vehicle treated group (0.31 ± 0.02 for wildtype mice vs 5.4 ± 0.87 for heterozygous mice, $P < 0.001$), which were further increased with stiripentol treatment (het mice + stiripentol) (Figure 2D, for wt, $N = 9$ mice for vehicle, STP or DZP groups, $N = 6$ mice for STP+DZP; for het, $N = 8$ for vehicle, STP, DZP and STP+DZP respectively, $P < 0.0001$ for interaction ($F(3,69) = 22.49$; for drug treatment ($F(3,69) = 26.33$ and for genotype ($F(1,69) = 73.40$)). With the pentylenetetrazol challenge, stiripentol alone increased total SWDs in the *Gabrg2^{+/Q390X}* mice (56.3 ± 6.9 , $P < 0.001$) while Diazepam alone (7.8 ± 1.66) or in combination with stiripentol (10.4 ± 2.1) reduced the total SWDs ($P < 0.0001$ for interaction ($F(3,57) = 22.17$; for drug treatment ($F(3,57) = 87.17$ and for genotype ($F(1,57) = 57.17$)). In Figure 2E, $P < 0.0001$ for interaction ($F(3,57) = 33.53$; for drug treatment ($F(3,57) = 49.53$ and for genotype) ($F(1,57) = 77.76$), Figure 2E). Similar to the findings for myoclonic jerks, the data indicated that STP increased SWDs which was reduced by DZP treatment. It is worth noting that the duration of ictal events in each respective group are not significantly different. The duration for myoclonic seizures was about 350 ms while the duration for SWD was about 3 sec across groups. Thus, the main difference of ictal events was the frequency in mice treated with different drugs.

DISCUSSION

The present study indicates that the polytherapy treatment of stiripentol and diazepam greatly attenuates seizure-related events in *Gabrg2^{+/Q390X}* knockin, a specific Dravet syndrome mouse. These findings are consistent with other studies suggesting the advantages of co-administering stiripentol with a benzodiazepine ((Fisher, 2011; Inoue and Ohtsuka, 2015)), and we are the first to report the effect of stiripentol in *Gabrg2^{+/Q390X}* mice. Stiripentol binds at a site on the $\alpha 3$ subunit that is specifically different from the benzodiazepine binding site, thus offering synergistic effects when stiripentol and a benzodiazepine are paired together (Chiron and Dulac, 2011). However, when stiripentol was administered alone or in conjunction with pentylenetetrazol, the ictal and interictal discharges associated with the *Gabrg2^{+/Q390X}* knockin mice were significantly enhanced. Our findings appear to be contradictory to other reports, which noted improved seizure control using only stiripentol as monotherapy (Cao et al., 2012; Chiron et al., 2000). For example, in a different Dravet syndrome mouse model *Scn1a^{+/R1407X}*, stiripentol alone has protective effects against hypothermia-induced seizures in mice at 1 month old but not in mice at 5 months old. It is possible that differential pathophysiology underlying each Dravet syndrome mouse model may contribute to the differential effect of stiripentol seen in each animal model. The main pathology of *Scn1a^{+/R1407X}* Dravet syndrome mouse is likely impaired GABAergic interneuronal activity, thus leading to reduced presynaptic GABA

release while the main pathophysiology of *Gabrg2^{+/Q390X}* Dravet syndrome mouse is reduced postsynaptic GABA_A receptor expression and channel function. Both pathologies in the two different conditions would give rise to a very similar clinical phenotype such as Dravet syndrome. Nevertheless, the effect of stiripentol for monotherapy merits further investigation as this information would be very valuable for clinical application but can not be tested directly in patients.

The mechanism of action for stiripentol still remains unclear, but it is reported to enhance the release of GABA as well as increase GABA_A receptor-mediated transmission (Quilichini et al., 2006). A key difference between the present study and other studies is the use of the *Gabrg2^{+/Q390X}* knockin mice. In *Gabrg2^{+/Q390X}* knockin mice, the nonsense mutation is known for producing nonfunctional properties at the $\gamma 2$ subunit (Kang et al., 2013), and it is possible there is altered GABAergic signaling, such as compensatory increase of GABA due to the presence of *Gabrg2(Q390X)* mutation, but this requires further elucidation. Given the importance of a properly functioning $\gamma 2$ subunit at the GABA_A receptor (Pritchett et al., 1989), the mechanism of action for stiripentol may have been altered to produce a different overall effect (i.e., enhancing spike-wave discharges) in *Gabrg2^{+/Q390X}* mice. However, this idea needs to be further explored by comparing DS models with different genetic mutations and thorough characterization of each model at the molecular level. It is worth noting that this study didn't use power spectrum analysis (Giordano et al., 2015), it is possible that some features went unnoticed during manual EEG scoring by which only a portion of data was sampled. Nevertheless, this study highlights the importance of achieving a mechanistic understanding and treatment for each group of mutations in different genes.

In conclusion, while not as beneficial when administered independently, the co-administration of stiripentol and diazepam reversed different types of seizures typically experienced in the *Gabrg2^{+/Q390X}* mice. Importantly, stiripentol alone increased survival during pentylenetetrazol seizure induction. This suggests that increased SWDs are not necessarily correlated with a higher mortality in epilepsy at least in genetic epilepsy with *GABRG2* deficiency based on this mouse model. Perhaps, the SWDs associated with STP in this particular disease model do not have the same effect as GTCSs that are associated with sudden unexpected death in epilepsy (SUDEP). The differences identified in this model to stiripentol treatment may be due to the pathogenic effect from *GABRG2(Q390X)* mutation (Kang et al., 2015), which may not be extrapolated to other Dravet syndrome mouse models. In summary, the findings from this study suggest that stiripentol may be beneficial for DS associated with *GABRG2* mutations when used in adjunct therapy. The findings also highlight the importance of understanding the etiologic heterogeneity for DS and mechanism-based precision treatment.

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REFERENCES

- Auvin S, Lecoite C, Dupuis N, Desnoux B, Lebon S, Gressens P, Dournaud P, 2013 Stiripentol exhibits higher anticonvulsant properties in the immature than in the mature rat brain. *Epilepsia* 54, 2082–2090. [PubMed: 24117113]
- Cao D, Ohtani H, Ogiwara I, Ohtani S, Takahashi Y, Yamakawa K, Inoue Y, 2012 Efficacy of stiripentol in hyperthermia-induced seizures in a mouse model of Dravet syndrome. *Epilepsia* 53, 1140–1145. [PubMed: 22578034]
- Chiron C, Dulac O, 2011 The pharmacologic treatment of Dravet syndrome. *Epilepsia* 52 Suppl 2, 72–75. [PubMed: 21463285]
- Chiron C, Marchand MC, Tran A, Rey E, d'Athis P, Vincent J, Dulac O, Pons G, 2000 Stiripentol in severe myoclonic epilepsy in infancy: a randomised placebo-controlled syndrome-dedicated trial. STICLO study group. *Lancet* 356, 1638–1642. [PubMed: 11089822]
- Fisher JL, 2011 Interactions between modulators of the GABA(A) receptor: Stiripentol and benzodiazepines. *Eur J Pharmacol* 654, 160–165. [PubMed: 21237147]
- Giordano C, Vinet J, Curia G, Biagini G, 2015 Repeated 6-Hz Corneal Stimulation Progressively Increases FosB/DeltaFosB Levels in the Lateral Amygdala and Induces Seizure Generalization to the Hippocampus. *Plos One* 10, e0141221. [PubMed: 26555229]
- Harkin LA, Bowser DN, Dibbens LM, Singh R, Phillips F, Wallace RH, Richards MC, Williams DA, Mulley JC, Berkovic SF, Scheffer IE, Petrou S, 2002 Truncation of the GABA(A)-receptor gamma2 subunit in a family with generalized epilepsy with febrile seizures plus. *Am J Hum Genet* 70, 530–536. [PubMed: 11748509]
- Hurst DL, 1990 Epidemiology of severe myoclonic epilepsy of infancy. *Epilepsia* 31, 397–400. [PubMed: 1695145]
- Inoue Y, Ohtsuka Y, 2015 Long-term safety and efficacy of stiripentol for the treatment of Dravet syndrome: A multicenter, open-label study in Japan. *Epilepsy Res* 113, 90–97. [PubMed: 25986195]
- Kang JQ, Shen W, Macdonald RL, 2009 The GABRG2 mutation, Q351X, associated with generalized epilepsy with febrile seizures plus, has both loss of function and dominant-negative suppression. *J Neurosci* 29, 2845–2856. [PubMed: 19261880]
- Kang JQ, Shen W, Macdonald RL, 2013 Trafficking-deficient mutant GABRG2 subunit amount may modify epilepsy phenotype. *Ann Neurol* 74, 547–559. [PubMed: 23720301]
- Kang JQ, Shen W, Zhou C, Xu D, Macdonald RL, 2015 The human epilepsy mutation GABRG2(Q390X) causes chronic subunit accumulation and neurodegeneration. *Nat Neurosci* 18, 988–996. [PubMed: 26005849]
- Pritchett DB, Sontheimer H, Shivers BD, Ymer S, Kettenmann H, Schofield PR, Seeburg PH, 1989 Importance of a novel GABAA receptor subunit for benzodiazepine pharmacology. *Nature* 338, 582–585. [PubMed: 2538761]
- Quilichini PP, Chiron C, Ben-Ari Y, Gozlan H, 2006 Stiripentol, a putative antiepileptic drug, enhances the duration of opening of GABA-A receptor channels. *Epilepsia* 47, 704–716. [PubMed: 16650136]
- Warner TA, Shen W, Huang X, Liu Z, Macdonald RL, Kang JQ, 2016 Differential molecular and behavioral alterations in mouse models of GABRG2 haploinsufficiency versus dominant negative mutations associated with human epilepsy. *Hum Mol Genet*.

Highlights

- The *Gabrg2^{+ / Q390X}* knockin mice associated with DS had increased seizures and death. (=85 characters)
- Stiripentol alone was ineffective in suppressing seizures but increased survival. (=82 characters)
- Add-on of stiripentol to diazepam was beneficial in *Gabrg2^{+ / Q390X}* mice. (=79 characters)
- Stiripentol can be used as adjunct therapy in DS with *GABRG2* deficiency. (=85 characters)
- The response to stiripentol may be different based on different genetic mutations. (=84 characters)

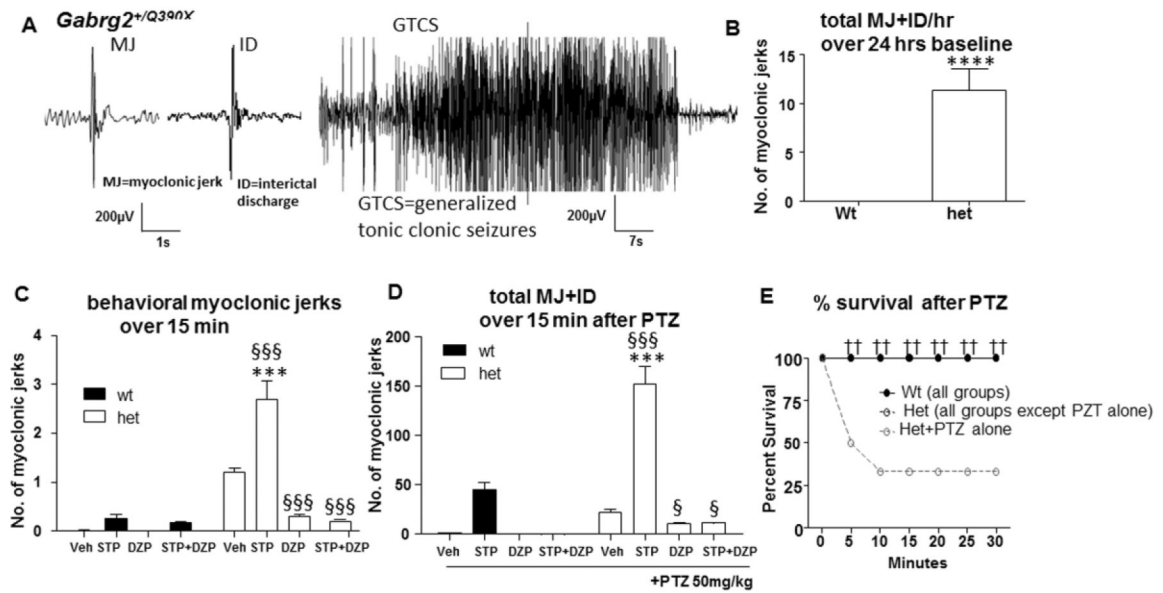


Figure 1. The effect of stiripentol on myoclonic ss and survival in *Gabrg2^{+/Q390X}* knockin mice. (A) Representative EEG recordings show that a 3-month old *Gabrg2^{+/Q390X}* knockin mouse had myoclonic jerks (Mj, left panel), interictal discharges (ID, middle panel) which is similar to myoclonic jerks in EEG but without overt behavioral correlation and generalized tonic clonic seizures (GTCS; right panel). (B) The frequency of MJ and ID in the wildtype (wt) or *Gabrg2^{+/Q390X}* heterozygous (het) mice was measured over a 24-hr session for baseline recording (n=15 for wt and 18 for het, n=15, ****P<0.0001, t=5.429, df=17, One sample t test with test). There was no injection involved in baseline recordings. The total numbers include events with behavioral jerks (MJ) and similar interictal discharges without overt behavioral manifestations (ID). (C) The frequency of behavioral myoclonic jerks in the wt or het mice was measured over the first 15 min of each hour after drug administration. Mice were treated with vehicle (0.9% normal saline, Veh, ip), stiripentol (150mg/kg, STP, ip), diazepam (0.3mg/kg, DZP, ip) alone or a combination of STP and DZP (N=8 for wt vehicle or STP, mice, N=10 for wt DZP, N=7 for STP+DZP; N=11 for het vehicle, STP, DZP and STP+DZP respectively; P<0.0001 for interaction (F(3,69)=22.19; for drug treatment) (F(3,69)=28.07 and for genotype (F(1,69)=73.01)); P<0.001 for both vehicle and STP treated het vs wt, Bonferroni posttests). (D) In pentylenetetrazol (PTZ) treated study, the frequency of total MJ and ID in the wt or het mice was measured over the 15 min after PTZ (50mg/kg, ip) administration (For wt, N=9 mice for vehicle, STP or DZP groups, N=6 mice for STP+DZP; for het, N=8 for vehicle, STP, DZP and STP+DZP respectively, P<0.0001 for interaction (F(3,57)=22.17; drug treatment (F(3,57)=87.17 and genotype (F(1,57)=57.17)); P<0.001 het vs wt for STP+PTZ, Bonferroni posttests. In both C-D, drug treatment and genotype are the independent variables, ***P<0.001 het vs wt with the same drug treated condition; §P<0.05, §§§P<0.001 vs het vehicle. Two-way ANOVA with Bonferroni posttests. The numbers represent the frequency of events with behavioral jerks and similar interictal discharges without overt behavioral manifestations. (E). Stiripentol alone, as well as in combination with DZP increased survival for *Gabrg2^{+/Q390X}* knockin mice during

pentylentetrazol (PTZ) seizure induction (N=12–15 for all wildtype and het mice except N=30 mice for het +PTZ group).

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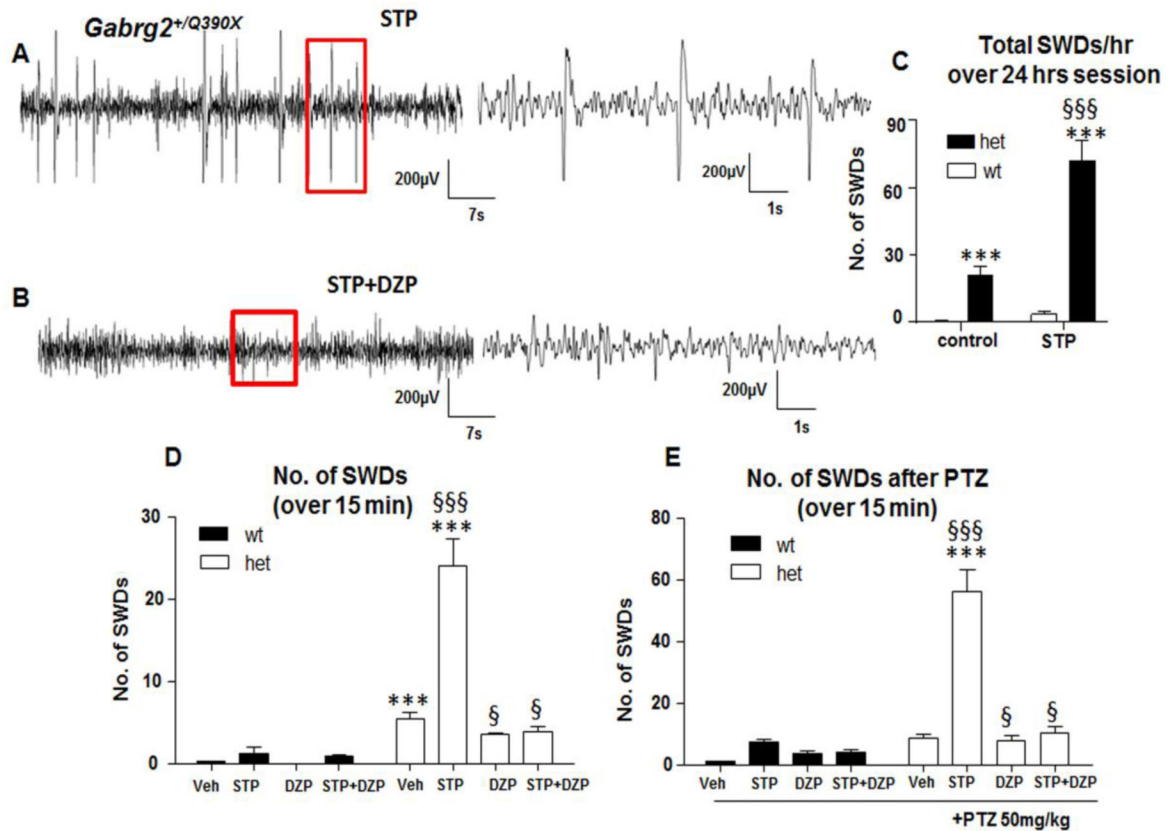


Figure 2. The effect of stiripentol on spike-wave discharges in *Gabrg2^{+/Q390X}* knockin mice. (A, B) Representative EEG recordings showing spike-wave discharges (SWDs) in a 3-month old *Gabrg2^{+/Q390X}* knockin mouse treated with stiripentol alone (STP, 150mg/kg, ip) (A) or with stiripentol plus diazepam (DZP, 0.3mg/kg, ip) (B). In A and B, boxed regions in the left panel were expanded in the right panel. (C) The occurrence of the SWDs in the wildtype (wt) or *Gabrg2^{+/Q390X}* heterozygous (het) mice was measured over a 24-hr session for baseline recording without any treatment or with stiripentol (N=15 for wt control, N=8 for wt STP; N=18 for het control and 8 for het STP, ***P<0.001 vs wt in control, §§§ vs het in control, genotype and treatment were taken as independent variables. P<0.001 for interaction F(1,43)=39.93; genotype F(1,43)=136.8 and for treatment F(1,43)=50.22, two-way ANOVA Bonferroni posttests). The first 10 min recordings of each hour were evaluated. (D) The total number of SWDs in the wt or het mice was measured over a 3-hr recording session. The first 15 min of each hour of recordings after drug administration (15 min after administration) was evaluated. Mice were treated with 0.9% normal saline (Vehicle, Veh), stiripentol (150mg/kg, STP, ip), Diazepam (0.3mg/kg, DZP, ip) alone or a combination of STP and DZP ((for wt, N=8 for vehicle and STP, 10 for DZP and 7 for STP+DZP, for het, N=11 for all four conditions. P<0.0001 for interaction (F(3,69)=22.49; drug treatment (F(3,69)=26.33 and genotype (F(1,69)=73.40)). (E) Stiripentol alone increased the SWDs in EEG in *Gabrg2^{+/Q390X}* knockin mice but DZP alone or in combination with stiripentol reduced the SWDs after a pentylenetetrazol (PTZ, 50mg/kg, ip) seizure induction (for wt, N=9 for vehicle, STP and DZP respectively and N=6 for STP+DZP; for het, N=8 for all four conditions, P<0.0001 for interaction (F(3,57)=33.53; for drug treatment (F(3,57)=49.53 and

for genotype) ($F(1,57)=77.76$). In **D** and **E**, $*P<0.05$, $***P<0.001$ vs wt with the same treated condition; $\$P<0.05$, $\$§§ P<0.001$ vs het vehicle. In **C, D, E**, drug treatment and genotype are the independent variables.

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