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Shift in interictal relative gamma power as a novel biomarker for drug response in two mouse models of absence epilepsy

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Summary

Objective—Two monogenic mouse models of childhood absence epilepsy, *stargazer* and *tottering*, differ strikingly in their response to *N*-methyl-D-aspartate (NMDA) receptor blockade. We sought to evaluate the change in interictal relative gamma power as a reliable biomarker for this gene-linked antiepileptic drug (AED) response.

Methods—The effects of AEDs on absolute and relative (to the total) power of frequencies between 2 and 300 Hz were analyzed within the interictal electroencephalogram (EEG) and correlated with antiseizure efficacy in awake behaving *stargazer*, *tottering*, and wild-type (WT) littermate control mice.

Results—At baseline, we found a significant absolute as well as relative augmentation of 16–41 Hz power in *stargazer* compared to both *tottering* and WT mice. In *stargazer,* the NMDA receptor–antagonist MK-801 (0.5 mg/kg) paradoxically exacerbates absence seizures but normalizes the augmented beta/gamma band of power to WT levels, suggesting that the elevation in 16- to 41-Hz power is an NMDA receptor–mediated network property. In contrast, ethosuximide (200 mg/kg) and 4-aminopyridine (2.5 mg/kg) reduce seizure activity and increase relative power within the gamma range in both *stargazer* and *tottering* mice. Intraperitoneal saline injection had no significant effect on either seizure frequency or relative gamma power. Along with results using carbamazepine and flupirtine, there was a strong inverse relationship between relative change in seizure duration and change in peak relative gamma power ($r^2 = 0.726$).

Significance—In these two models of absence epilepsy, drugs that reduce relative gamma power are associated with an increase in seizures, whereas drugs that augment relative gamma power reduce seizures. Therefore, drug-induced modulation of relative gamma power may serve as a biomarker for AED efficacy in absence epilepsy. Given the relationship between gamma power

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and fast-spiking interneurons, these results also suggest that a drug's effect may in part be determined by its impact on specific inhibitory networks.

Keywords

stargazer; *tottering*; Interneurons; NMDA receptors; Parvalbumin

Childhood absence epilepsy is a form of idiopathic (genetic) generalized epilepsy characterized by ictal 3- to 4-Hz generalized spike-and-wave activity in the electroencepahlogram (EEG) associated with behavioral arrest. Ethosuximide, the first-line medication for absence epilepsy, controls seizures in only 45% of patients and is otherwise either ineffective or intolerable.[1] Commonly used antiepileptic drugs (AEDs) including carbamazepine and phenytoin are usually ineffective and can even paradoxically aggravate absence seizures.[2] Unfortunately, in epilepsy therapy in general, there are no well-established interictal biomarkers that predict whether medication will be effective, and a biomarker that could provide insight into whether an AED might aggravate seizures in a patient would be of particular clinical importance.

Single-gene mouse models of absence epilepsy provide a valuable opportunity to explore the pharmacogenetics of inherited epilepsies, and may ultimately help identify genes that could guide therapy. More than 20 genes linked to the appearance of spike-wave seizures have been identified,[3] and, all of these models are sensitive to ethosuximide. Recently, however, gene-linked differences in response to other antiepileptic drugs in these models have been described.[4] The *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801, although not used clinically, is effective at treating seizures in the *tottering* mouse model of absence epilepsy, whereas it causes a robust seizure exacerbation in *stargazer* mice, despite having the shared phenotype of absence epilepsy.[4] One biologic mechanism that may be responsible for this striking difference in pharmacologic response is that the genetic defect in *stargazer* mice impairs trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors to the dendrites of parvalbumin-expressing (PV+) interneurons, leaving NMDA receptors as the major excitatory receptor to trigger these cells.[4] Blocking NMDA receptors in *stargazer* mice may further cripple the normal activation of PV+ interneurons, leading to stronger disinhibition and aggravation of seizures. In contrast, *tottering* mice have a genetic mutation in the P/Q-type calcium channel, which has been implicated in defective presynaptic γ-aminobutyric acid (GABA) release from neocortical PV+ interneurons.[5] Therefore, *tottering* mice should have preserved AMPA receptor function in PV+ interneurons, which may explain the lack of paradoxical seizure aggravation with NMDA-receptor antagonism in that model.

Because PV+ interneurons not only provide a powerful means of inhibiting epileptic seizures, but have also been linked to gamma power in cortical neuronal networks,[6] a biomarker for drug efficacy may be hidden at high frequencies in the EEG. EEG frequencies at ≥30 Hz have historically been divided into different bands, but they can generally be grouped together under the umbrella of "high frequency oscillations," or HFOs.[7] PV+ interneurons have been linked to the generation of oscillations in the 30- to 200-Hz range, including neocortical gamma oscillations (30–100 Hz),[6,8] neocortical ripples (100–200 Hz),[9]

and the overlap between the two (50–200 Hz).[10] Neocortical interneurons fire synchronously with these fast oscillations,[11] supporting their role in generating both gamma and ripple activity.[12] Oscillations >200 Hz (fast ripple oscillations) have been associated with pathologic epileptiform discharges in epilepsy with both focal-[13] and generalized-[14] onset seizures.

Because fast oscillations can be generated by fast-spiking, PV+ interneurons, and *stargazer* mice have a mutation that may reduce the excitatory synaptic drive onto these neurons, we examined the dynamics of absolute and relative gamma power in *stargazer*, *tottering*, and wild-type (WT) mice at baseline and in response to drugs that have variable effects on seizures.

Methods

Mice

Experiments used adult homozygous *stargazer* (*stg/stg*) mutants and adult homozygous *tottering* (*tg/tg*) mutants (>6 weeks old) on a C57BL6/J background and their WT littermate controls (+/+) of either sex. Genotypes were confirmed by polymerase chain reaction (PCR) of tail DNA. All animal research was performed in accordance with Baylor College of Medicine Institutional Animal Care and Use Committee (IACUC) guidelines and regulations.

In vivo video-EEG monitoring

Mice were anesthetized by Avertin (20 μl/g, i.p.) and surgically implanted with bilateral silver wire electrodes (0.005-inch diameter) inserted into the epidural space over the somatosensory cortex (1 mm posterior and 3 mm lateral to bregma) bilaterally through cranial burr holes and attached to a microminiature connector cemented to the skull. Mice were allowed to recover for at least 2 weeks prior to recording. EEG and behavioral activity in freely moving mice were recorded using simultaneous video-EEG monitoring (Harmonie software version 6.1c, Stellate Systems). All in vivo experiments were performed between 12 p.m. and 3 p.m. to prevent confounding diurnal variation. All EEG signals were sampled at 2 kHz with an antialiasing filter and then notch filtered with a 1 Hz window around 60 Hz, 120 Hz, 180 Hz, and 240 Hz using EEGLab[15] in MATLAB (Mathworks, Inc). Mice were allowed to acclimate to the recording environment for 30 min, and video-EEG was then collected for a 30-min baseline sampling period, followed by intraperitoneal drug injection. Drug effect was analyzed between 30 and 60 min after drug administration. Recordings were excluded if the EEG was contaminated by artifact. Investigators were blinded to genotype and drug administered prior to analysis. Seizure activity was defined by bilateral spike and wave discharges with amplitude ≥1.5× baseline voltage and concomitant video-recorded behavioral arrest. Because the power of HFOs may be falsely measured when there are sharp contours,[16,17] seizure episodes were digitally extracted in EEGLab[15] to evaluate only interictal activity periods. Total seconds of seizure activity, independent of the number of seizure episodes or their duration, were summed and compared to the pre-drug baseline seizure duration (paired *t*-test, significance set at $p < 0.05$). Pre-and postdrug interictal EEG data were then analyzed using the spectral analysis function [spectropo()] in EEGLab.[15] The

recordings were analyzed for power between 2 and 300 Hz, giving an output for absolute power (AP) in dB ($\log_{10}$ (μV^2/Hz)) for 154 points at 1.95 Hz intervals. Relative power (RP) was calculated by dividing the absolute power for each frequency by the total power (TP), and then normalized with a log transformation before comparison between mice (RP=AP/TP), similar to methods described previously.[18,19] Statistical differences between groups at baseline were tested using a two-way analysis of variance (ANOVA) with Bonferroni posttests at each frequency. Differences due to drug exposure were tested using a repeated-measures two-way ANOVA to compare groups before and after drug administration with Bonferroni post-tests at each frequency. Statistical significance was set at $p < 0.05$ at two or more consecutive frequencies. Change in relative seizure duration was plotted against peak change in relative gamma power for each drug to determine a cumulative correlation coefficient (r^2). All statistical analysis was performed using Prism 5, version 5.0d, GraphPad, CA.

Drugs

Drugs used were ethosuximide (Sigma-Aldrich), MK-801 (Tocris), flupirtine (Selleck Chemicals), carbamazepine (AcrŌs Organics), and 4-aminopyridine (4-AP) (Sigma-Aldrich). Ethosuximide, flupirtine, and carbamazepine were first dissolved in dimethyl sulfoxide (DMSO) prior to being brought to 1% volume/weight of each animal being tested in a phosphate-buffered saline solution (Gibco, Life Technologies).

Results

Baseline absolute and relative beta/gamma power is augmented in *stargazer* compared to both *tottering* and WT mice

Stargazer mice (n = 12) had significantly greater baseline interictal AP in the beta/gamma range compared to WT mice (n = 10, 14–47 Hz, $p < 0.05$; maximal at 18 Hz, $p < 0.0001$) as well as *tottering* mice (n = 11, 16–49 Hz, $p < 0.05$; maximal at 35 Hz, $p < 0.001$). RP in *stargazer* mice (n = 12) was elevated in the beta/gamma range compared to WT (n = 10, 16–41 Hz, $p < 0.05$; maximal at 18 Hz in the beta range, $p < 0.0001$, and at 31 Hz in the gamma range, $p < 0.01$) and compared to *tottering* mice (16–49 Hz, $p < 0.05$; maximal at 29 Hz in the beta range, $p < 0.001$, and at 35 Hz in the gamma range, $p < 0.001$), but there was no significant difference in either AP or RP between *tottering* and WT mice at any frequency (Fig. 1). These results demonstrate that *stargazer* mice have augmented interictal beta and gamma range power at baseline.

MK-801 has an opposite effect on both seizures and relative gamma power in *stargazer* and *tottering* mice

The elevated gamma power in *stargazer* mice suggests hyperactivity of parvalbumin interneurons as seen in the thalamic reticular nucleus due to enhanced NMDA-receptor activation.[20] To determine if the augmentation in neocortical 16–41 Hz power in *stargazer* mice might be sustained due to compensation for the dendritic AMPA-receptor defect by secondary NMDA-receptor activation, 0.5 mg/kg MK-801, i.p., a noncompetitive NMDA receptor (NMDAR) antagonist, was administered. As seen previously,[4] MK-801 caused seizure exacerbation with irregular 3- to 4-Hz spike-wave discharges in *stargazer* mice

(mean ± standard error of the mean (SEM), 3.46x ± 0.86x relative seizure duration, n = 6, p = 0.0002). MK-801 also caused a trend toward reduction in absolute beta/gamma power (maximal at 31 Hz) and a significant reduction in relative beta/gamma power to WT levels (18–39 Hz, n = 6, $p < 0.05$; maximal at 31 Hz, $p < 0.001$) (Fig. 2A). In *tottering* mice (n = 5), MK- 801 significantly reduced seizures (0.22x ± 0.15x, p = 0.041) and caused an increase in absolute gamma (72–106 Hz, $p < 0.05$, maximal at 84 Hz, $p < 0.01$) and ripple (152–180 Hz, $p < 0.05$, maximal at 164 Hz, $p < 0.0001$) power. There was a parallel increase in RP in the gamma (70–111 Hz, $p < 0.05$; maximal at 84 Hz, $p < 0.001$) and ripple (152–180 Hz, $p < 0.05$; maximal at 168 Hz, $p < 0.0001$) range (Fig. 2B). In WT mice (n = 8), there was a similar elevation in absolute (143–182 Hz, $p < 0.05$; maximal at 162 Hz, $p < 0.0001$) and relative (148–178 Hz, $p < 0.05$; maximal at 162 Hz, $p < 0.0001$) ripple power, but no significant change was found in AP or RP in *stargazer* mice at these frequencies (Fig. 2B). Post hoc comparison of *stargazer* post-MK-801 and WT post-saline showed no significant difference at any frequency (Fig. 2A, two-way ANOVA, $p > 0.05$). These results demonstrate that drug-induced seizure exacerbation may be associated with reduction of relative gamma power, whereas seizure reduction due to effective treatment may be associated with an increase in relative gamma power.

Flupirtine aggravates seizures and reduces interictal relative gamma power in *tottering* but not *stargazer* mice

In a screen for other drugs that exacerbate seizures in these two models, we found that 10 mg/kg flupirtine significantly aggravated seizures in *tottering* mice (mean ± SEM, 3.64x ± 0.81x relative seizure duration, n = 6, p = 0.003) yet had no significant effect on seizures in *stargazer* mice (1.22x ± 0.09x, n = 5, $p > 0.05$). AP was increased in *tottering* mice between 10 and 25 Hz and reduced between 59 to 66 Hz as well as 100–119 Hz. RP was similarly increased between 10 and 21 Hz and reduced broadly between 51 and 166 Hz in *tottering* mice (n = 6, $p < 0.05$; maximal at 61 Hz in the gamma range, $p < 0.0001$ and at 115 Hz in the ripple range, $p < 0.0001$). WT mice, similar to *tottering* mice, had a significant increase in AP between 16 and 18 Hz and a decrease between 100 and 166 Hz, while RP was increased between16–18 Hz and decreased between 59 and 182 Hz (n = 6, $p < 0.05$; maximal at 16 Hz, $p < 0.05$, and 132 Hz, $p < 0.0001$, respectively). There was no significant change in AP or RP at any frequency in *stargazer* mice with 10 mg/kg flupirtine (n = 5, $p > 0.05$) (Fig. 3). Therefore, the effect of flupirtine in *tottering* was similar to the effect of MK-801 in *stargazer* in that a significant reduction in relative gamma power showed a consistent link to seizure exacerbation.

Ethosuximide and 4-aminopyridine (4-AP) reduce seizures and increase relative gamma power

For comparison with AEDs that are effective for absence seizures in both models, we examined the effect of ethosuximide and 4-AP. Intraperitoneal ethosuximide (200 mg/kg) was administered in *stargazer* (n = 7), *tottering* (n = 6), and WT (n = 8) mice (Fig. 4A). Ethosuximide caused a significant reduction in absolute power in the fast ripple range between 186 and 300 Hz in *stargazer* mice ($p < 0.05$; maximal at 283 Hz, $p < 0.0001$), between 191 and 300 Hz in *tottering* mice ($p < 0.05$; maximal at 287 Hz, $p < 0.0001$), and between 170 and 221 Hz in WT mice ($p < 0.05$; maximal at 201 Hz, $p < 0.01$). Absolute

power in other frequency bands was largely unaffected, with the exception of a significant reduction in AP between 10 and 14 Hz with ethosuximide in WT mice ($p < 0.05$; maximal at 12 Hz, $p < 0.01$) and a significant reduction between 2 and 21 Hz ($p < 0.05$; maximal at 2 Hz, $p < 0.0001$) in *tottering* mice. When corrected for TP, in contrast to AP, there was a significant increase in RP between 51 and 55 Hz power in *stargazer* mice ($p < 0.05$; maximal at 53 Hz, $p < 0.05$), between 66 and 115 Hz in *tottering* mice ($p < 0.05$; maximal at 92 Hz, $p < 0.001$), and between 31 and 141 Hz in WT mice ($p < 0.05$; maximal at 51 Hz, $p < 0.0001$) (Fig. 4A).

Because 4-AP has been shown previously to be antiepileptic in *tottering* mice at doses of 1–5 mg/kg,[21] we administered 2.5 mg/kg 4-AP in *stargazer* mice, which caused a similar significant reduction in seizures (mean ± SEM, 0.21x ± 0.10x, n = 5, p = 0.011). We also confirmed a significant reduction in seizures in *tottering* mice (0.09x ± 0.08x, n = 6, p = 0.017). In *stargazer* mice, there was a significant increase in AP between 57 and 59 Hz, and a significant increase in RP between 53 and 68 Hz (n = 5, $p < 0.05$; maximal at 59 Hz, $p < 0.0001$, Fig. 4B). In *tottering* mice, there was a similar significant increase in AP between 33 and 102 Hz (n = 6, $p < 0.05$; maximal at 74 Hz, $p < 0.0001$) with a significant reduction in AP between 209 and 300 Hz ($p < 0.05$; maximal at 295 Hz, $p < 0.0001$). RP, however, was broadly increased between 25 and 143 Hz in *tottering* mice ($p < 0.05$; maximal at 74 Hz, $p < 0.0001$, Fig. 4B). WT mice had no significant change in AP or RP at any frequency (n = 5, $p > 0.05$), although there was a trend toward an increase in relative gamma power, maximal at 35 Hz (Fig. 4B). Taken together, administration of ethosuximide and 4-AP showed that effective treatment of seizures in both models of absence epilepsy may be associated with an increase in relative, but not necessarily absolute, gamma power.

Carbamazepine has no significant effect on seizure activity or relative power in *stargazer* and *tottering* mice

In WT mice (n = 6), 20 mg/kg carbamazepine caused a significant increase in AP between 2 and 6 Hz ($p < 0.05$; maximal at 4 Hz, $p < 0.001$) and a significant decrease in AP between 68 and 152 Hz ($p < 0.05$; maximal at 137 Hz, $p < 0.01$, Fig. 5A). In contrast, AP was significantly decreased between 176 and 300 Hz in *tottering* mic (n = 5, $p < 0.05$; maximal at 291 Hz), and there was no change in AP at any frequency for *stargazer* mice (n = 6, $p > 0.05$, Fig. 5A).

With regard to RP, WT mice had a significant broad reduction between 31and 190 Hz ($p < 0.05$; maximal at 137 Hz, $p < 0.0001$). Neither *stargazer* nor *tottering* mice had a significant change in RP at any frequency (Fig. 5B).

Therefore, despite causing a significant reduction in relative gamma power in WT mice, carbamazepine did not affect seizures or relative gamma power in either model of absence epilepsy.

Composite shift in peak relative gamma power is inversely correlated with the postdrug change in seizure duration

To evaluate the relationship between change in relative gamma power and change in seizure duration postdrug, a data point representing these two variables was created for each drug

given for each genotype (Fig. 6). In general, drugs that reduced relative gamma power were associated with an increase in seizures; drugs that augmented relative gamma power were associated with a reduction in seizures; and drugs (including saline) that had no significant effect on relative gamma power had no significant effect on seizure activity ($n = 69$, $r^2 = 0.726$).

Discussion

In this study we examined the relationship between the effect of AEDs on interictal gamma power and seizures in two distinct monogenic mouse models of absence epilepsy. Despite the AMPA-receptor trafficking deficit localized to neocortical PV+ interneurons,[4] baseline interictal gamma power was augmented in *stargazer* mice compared to WT mice, and abolished with NMDA-receptor antagonism. We also found that the shift in interictal relative gamma power correlated strongly with AED efficacy in both *stargazer* and *tottering* models.

Baseline elevation in gamma power in *stargazer* mice

Given the reduction in AMPA-receptor trafficking in PV+ interneurons in the somatosensory cortex of *stargazer* mice and the reported importance of fast-spiking interneuron activation for maintaining gamma oscillations,[8] we predicted a reduction in relative gamma power in *stargazer* mice. However, interictal gamma power was significantly elevated in *stargazer* compared to both *tottering* and WT mice. This finding is nevertheless consistent with augmented gamma power (30–100 Hz) previously found in the interictal EEG of primary generalized epilepsies in humans[22] as well as the Genetic Absence Epilepsy in Rats from Strasbourg (GAERS) rat model of absence epilepsy.[23] In contrast, we found that *tottering* mice have normal baseline gamma power. Therefore, absence epilepsy is not necessarily associated with augmented baseline gamma power.

The unique advantage of the dichotomy in gamma power elevation in *stargazer* and *tottering* mice is that this difference can be linked to single genes, which may provide mechanistic insights into the seizure network. Because the mutation in *stargazer* impairs AMPA-receptor trafficking in neocortical PV+ interneurons,[4] the elevation (rather than the expected depression) in interictal gamma power suggests a secondary compensatory response mediated by NMDA receptors on PV+ interneurons, or other changes in network excitability. The former mechanism is supported by the enhanced NMDA receptor–mediated current previously found on parvalbumin+ inhibitory interneurons in the reticular thalamic nucleus of *stargazer* mice.[20]

NMDA-receptor antagonism reduces interictal relative beta and gamma power to WT levels and fails to increase ripple oscillations in *stargazer* mice

To test if the elevation in interictal gamma power could be mediated by NMDA receptors on PV+ interneurons, we challenged *stargazer, tottering*, and WT mice with an NMDA-receptor antagonist. Although MK-801 caused seizure exacerbation in *stargazer* mice as reported previously,[4] NMDA-receptor blockade normalized the augmented interictal relative gamma power in *stargazer* mice. This finding gives more support to a postsynaptic plasticity model invoking NMDA receptor–mediated activation of PV+ interneurons as a homeostatic

compensation for reduced dendritic AMPA-receptor trafficking. However, further studies are required to determine whether NMDA receptors are specifically upregulated in PV+ interneurons in *stargazer* mice.

In rats, NMDA-receptor antagonists typically cause an increase in gamma (30–100 Hz) and ripple (100–200 Hz) power.[25] The effect of NMDA-receptor antagonists on frequencies >100 Hz in mouse neocortex, however, has not been extensively studied. An elevation in ripple power was seen in both WT and *tottering* mice but was absent in *stargazer* mice (Fig. 2B), presumably because AMPA receptor–mediated activation of PV+ interneurons is critical for maintaining and inducing ripple oscillations.[13]

Ethosuximide and 4-aminopyridine reduce absence seizures and increase relative gamma power

Ethosuximide is the first-line therapy for childhood absence epilepsy[1] and causes no significant change in AP at frequencies traditionally visible in the scalp EEG (up to 30 Hz). The effect of ethosuximide on higher interictal EEG frequencies has not previously been evaluated in detail. Here we found that it reduced absolute interictal fast ripple power in both *stargazer* and *tottering* mice, which may be due to reduced desynchronized 200- to 500-Hz bursting mediated by T-type calcium channels.[25] Although most of the EEG signal is considered to arise from summated slow excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs), there can be “spectral leakage” from bursting activity of cortical neurons.[17] Ethosuximide caused no significant change in absolute gamma power but increased relative gamma power in *stargazer*, *tottering*, and WT mice (Fig. 4). These findings are consistent with a developmental downregulation of T-type calcium channels on fast-spiking interneurons.[26] However, the mechanism by which ethosuximide reduces total power to increase relative gamma power remains unclear.

Paradoxically, a subconvulsive dose of 2.5 mg/kg 4-AP also significantly reduced seizures in both *stargazer* and *tottering* mice and was associated with a significant increase in both absolute and relative gamma power. WT mice had a nonsignificant increase in relative gamma power at the same dose, suggesting that the effect of 4-AP on inhibitory networks may be enhanced in *stargazer* and *tottering* mice. 4-AP blocks potassium channels in both excitatory and inhibitory neurons, causing overall network hyperexcitability.[27] Deleting Kv3.1 (expressed primarily in PV+ interneurons) in mice has also been shown to significantly increase 20- to 60-Hz gamma oscillatory power,[28] and 4-AP increases the duration of action potentials of neocortical fast-spiking interneurons.[29] Therefore, upregulation of potassium channels in fast-spiking interneurons could be one potential mechanism for the pharmacogenetic efficacy of 4-AP in *stargazer* and *tottering* mice.

Carbamazepine reduces absolute and relative gamma power in WT mice, but does not significantly change relative gamma power or seizures in *stargazer* or *tottering* mice

Although 20 mg/kg carbamazepine had no significant effect on seizures or relative power at any frequency in both mutant models, it did reduce relative gamma power in WT mice (Fig. 5). In contrast to our results, oral administration of carbamazepine at 25 mg/kg has been shown to significantly aggravate seizures in *stargazer* mice.[30] Seizure exacerbation with

carbamazepine has also been shown in the GAERS[31] and Wistar Albino Glaxo rats from Rijswijk (WAG/Rij)[32] rat models of absence epilepsy at doses between 20 and 40 mg/kg. The reason for the lack of seizure exacerbation in both models in our study is unclear, but may be related to the drug's dose or pharmacodynamic properties. However, the concomitant lack of change in relative gamma power is consistent with the effect of all other drugs administered in this study.

Drug-induced change in relative interictal gamma power is inversely correlated with absence seizures in two mouse models

Gamma power, along with high frequency oscillatory power, is generally elevated with focal neocortical seizure activity,[11] and has been investigated as a potential biomarker for the seizure-onset zone.[33] In contrast, there is a strong inverse association between absence seizures and interictal relative gamma activity, suggesting independent mechanisms for epileptogenesis. These findings are consistent with the distinct clinical presentation and EEG signatures in focal-onset versus generalized-onset seizures. Neither baseline interictal EEG power nor a change in absolute gamma power showed a correlation with AED efficacy; only the drug-induced change in relative gamma power consistently predicted outcome in these models of absence epilepsy. These findings agree with those of a prior study in the inbred Dilute Brown Agouti from Jackson Laboratories (DBA/2J) mouse model of absence epilepsy in which seizure aggravation with baclofen was associated with a reduction in interictal gamma power.[34] The mechanism behind this relationship is unclear, but one potential explanation may lie in predisposing deficits in inhibitory networks. Specifically, dysfunction in feedforward inhibition has been implicated in a number of monogenic models of absence epilepsy,[3] and gamma oscillations have been shown to arise as a network property of intact feedforward inhibition.[35] *Tottering* mice, with a point mutation in *Cacna1a*, have deficits in thalamocortical feedforward inhibition,[36] and yet they have a baseline gamma power equivalent to that of WT mice. This may be secondary to the relatively mild severity of the mutation, since deletion of *Cacna1a* causes absence status epilepticus and a dramatic reduction in baseline gamma band activity.[37] Compensatory changes during development, therefore, might normalize gamma power or paradoxically enhance baseline gamma power as seen in *stargazer*. A dependence on NMDA receptor–mediated compensation may in turn render gamma oscillations selectively vulnerable to NMDA-receptor blockade. However, this mechanism of drug effect on gamma oscillations in absence epilepsy remains speculative and warrants further investigation.

There are challenges with translating gamma power shifts in response to AEDs in patients with absence epilepsy, since these patients are not implanted with intracranial electrodes. However, measurements of oscillations up to 200 Hz from scalp EEG recordings have been validated previously,[38] and changes in ripple-band activity were recently evaluated in the treatment of West syndrome, another generalized epilepsy syndrome.[39] In addition, it is unlikely that clinicians will use interictal gamma power as a biomarker for drug effect in patients with childhood absence epilepsy given that seizures are commonly captured on routine EEG. In future experiments, it will be interesting to study other types of epilepsy that have been associated with fast-spiking interneuronopathies and have less frequent seizures, such as Dravet syndrome,[40] for biomarkers within the gamma range of the EEG.

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Biography

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Key Points

- There is a gene-linked baseline augmentation of beta and gamma power in *stargazer* mice compared to both *tottering* and WT mice
- NMDA-receptor blockade leads to seizure exacerbation in *stargazer* but not *tottering* mice and normalizes the power spectrum to WT levels
- In both absence models, the shift in peak interictal relative gamma power is inversely correlated with a drug's effect on seizures
- Relative gamma power may serve as a predictive biomarker for AED efficacy in generalized spike-wave epilepsy

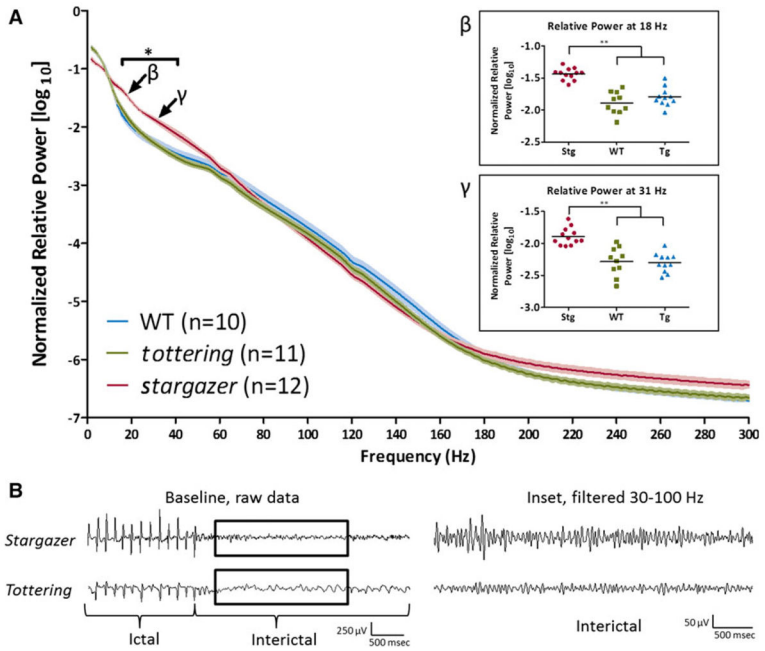

Figure 1.

Baseline differences in interictal gamma power. (A) Mean ± standard error of the mean (SEM) of relative power between WT, *tottering*, and *stargazer* from 2 to 300 Hz. There is a baseline augmentation of relative beta and gamma power (16–37 Hz) in *stargazer* compared to both WT and *tottering* mice (*p < 0.01). Inset: distribution of relative power at 18 Hz (peak beta power difference) and 31 Hz (peak gamma power difference) between groups (arrows) (**p < 0.01, corrected for multiple comparisons). (B) Example of raw interictal EEG sampled at 2 kHz (left) with representative traces filtered between 30 and 100 Hz (inset, right).

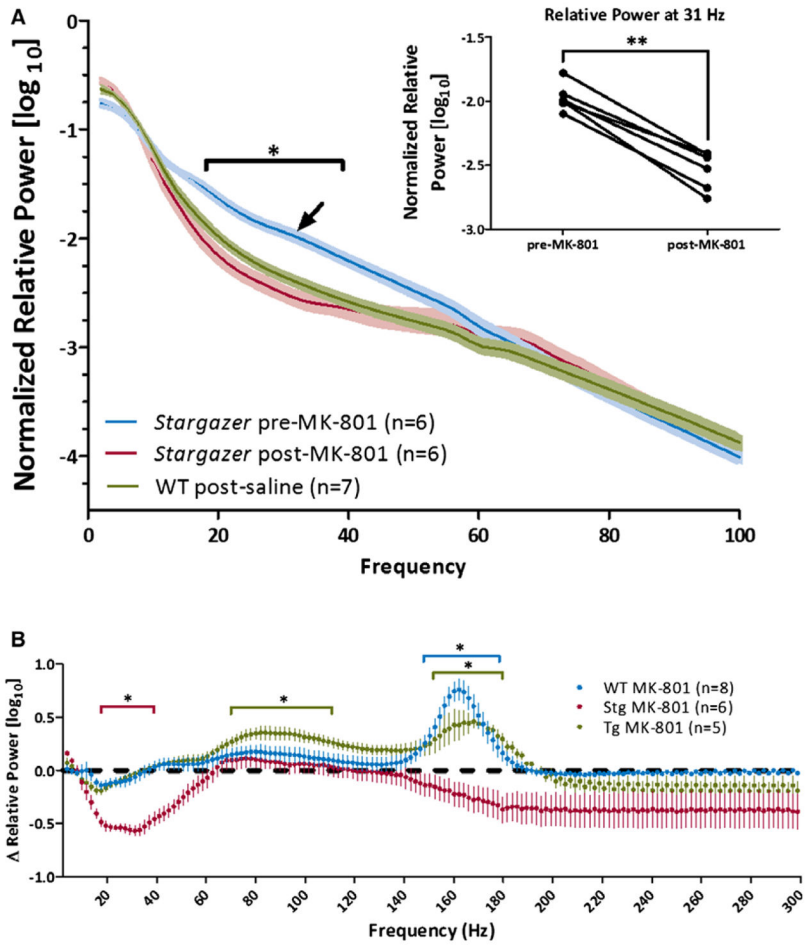

Figure 2.

Response to NMDA-receptor blockade (mean ± SEM) (A) Recovery of augmented beta/gamma power (18–39 Hz) following 0.5 mg/kg MK-801 injection in *stargazer* mice (*$p < 0.05$). The maximum difference was at 31 Hz (arrow/inset, **$p < 0.001$). (B) Elevation in relative power between 148 and 178 Hz ($p < 0.05$) in WT mice ($n = 8$) and between 70–111 Hz and 152–180 Hz ($p < 0.05$) in *tottering* mice ($n = 5$), compared to significant reduction in beta/gamma power in *stargazer* mice ($n = 6$, $p < 0.05$).

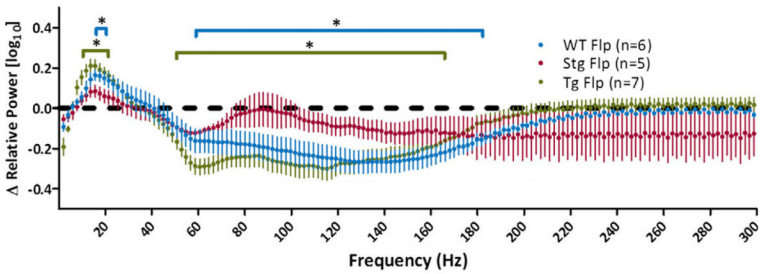

Figure 3.

Flupirtine 10 mg/kg in *tottering* mice caused a significant increase in relative power in the alpha/beta range (10–21 Hz) and a significant decrease in the gamma/ripple range (mean ± SEM, 51–166 Hz, n = 7, *p < 0.05). In contrast, *stargazer* mice had no significant change at any power (n = 5, p > 0.05).

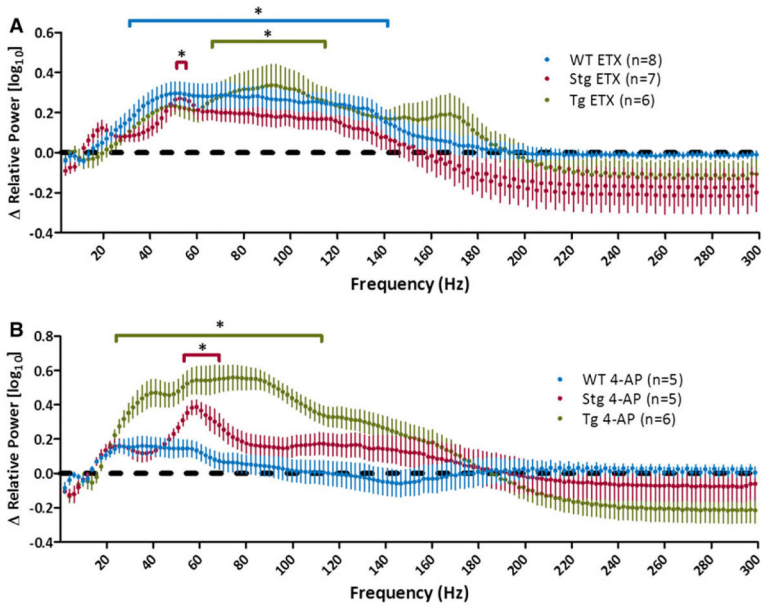

Figure 4.

(A) Significant increase in relative gamma power with 200 mg/kg ethosuximide in WT, *stargazer*, and *tottering* mice. (B) 4-Aminopyridine significantly increased relative gamma power in *stargazer* and *tottering* mice, but not in WT mice (mean ± SEM, *p < 0.05).

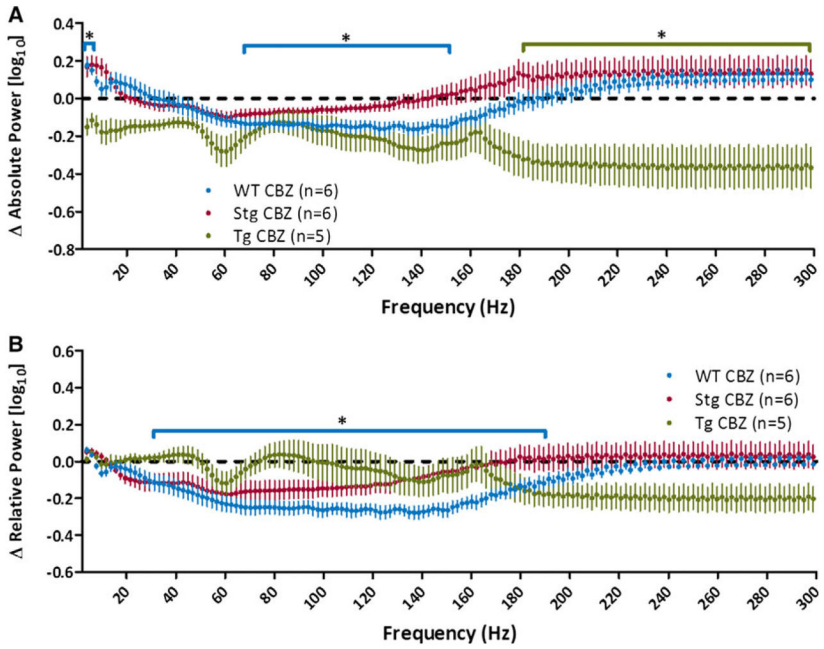

Figure 5.

Changes with 20 mg/kg carbamazepine in absolute (A) and relative (B) power (mean ± SEM). In wild-type mice, there was a significant reduction in both absolute and relative gamma/ripple range power. *Tottering* mice had a significant reduction in absolute fast ripple power but neither *stargazer* nor *tottering* had any significant reduction in relative power at any frequency.

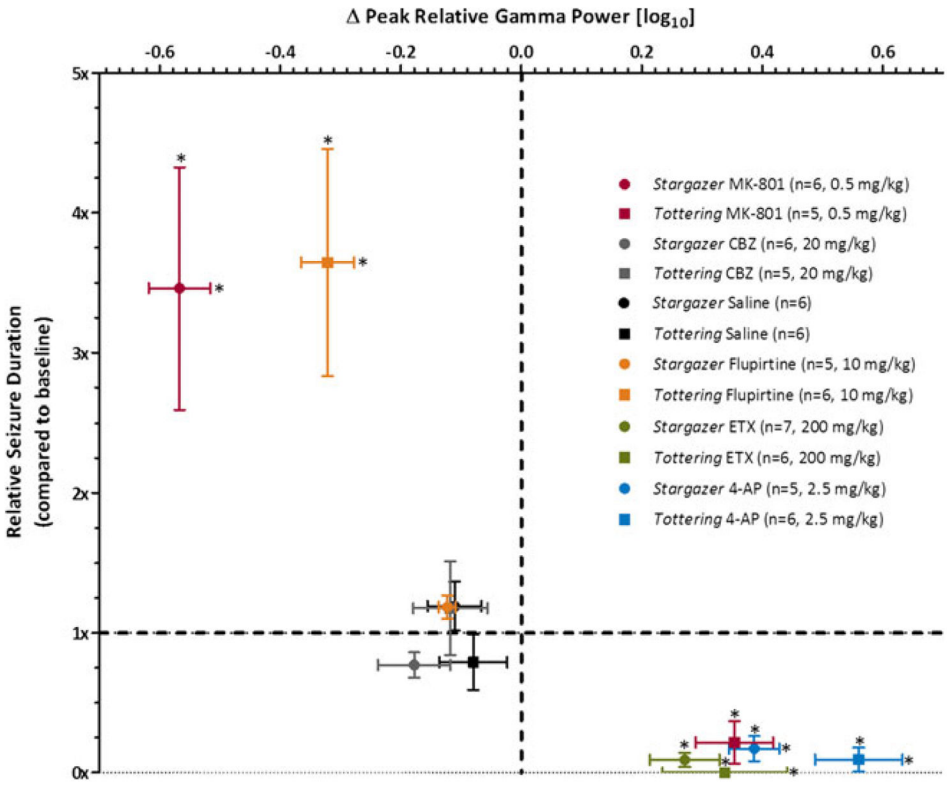

Figure 6.

Inverse correlation between AED efficacy (mean ± SEM, *p < 0.05) and change in peak relative gamma power (mean ± SEM, *p < 0.05). Drug responses cluster into three groups based on relative seizure duration. Seizure aggravation (top) is associated with reduction in relative gamma power, whereas seizure reduction (bottom) is associated with augmented relative gamma power. Drugs with no significant effect on seizures (middle) have no significant effect on gamma power. Overall, there is an inverse correlation between the mean change in relative gamma power and mean change in seizure duration for a given drug (r^2 = 0.726). Gene-linked differences between *stargazer* (circles) and *tottering* (squares) mice are apparent with administration of MK-801 (red) and flupirtine (orange).