Interictal relative gamma power as a biomarker for anti-epileptic drug response in absence epilepsy

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Introduction

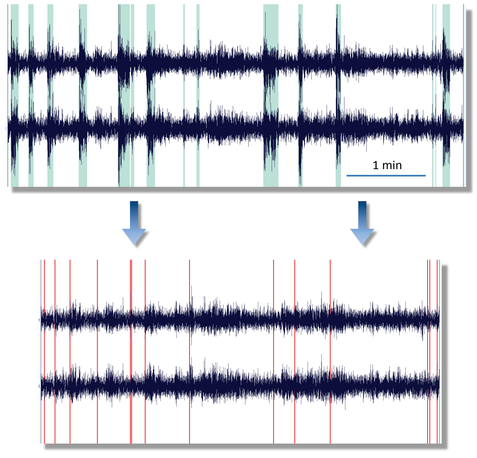
Childhood absence epilepsy is a form of idiopathic (genetic) generalized epilepsy characterized by ictal 3-4 Hz generalized spike and wave activity on the electroencephalogram (EEG) and behavioral arrest. Medications including carbamazepine and phenytoin are usually ineffective and can even paradoxically aggravate absence seizures[1].

The NMDA receptor antagonist MK-801, while 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[2]. This effect in *stargazer* mice may be due to mis-trafficking of AMPA receptors to the dendrites of parvalbumin-expressing (PV+) interneurons, leaving NMDA receptors as the major excitatory receptor to trigger these cells[2]. Blocking NMDA receptors in *stargazer* mice may further cripple activation of PV+ interneurons, leading to stronger disinhibition and aggravation of seizures.

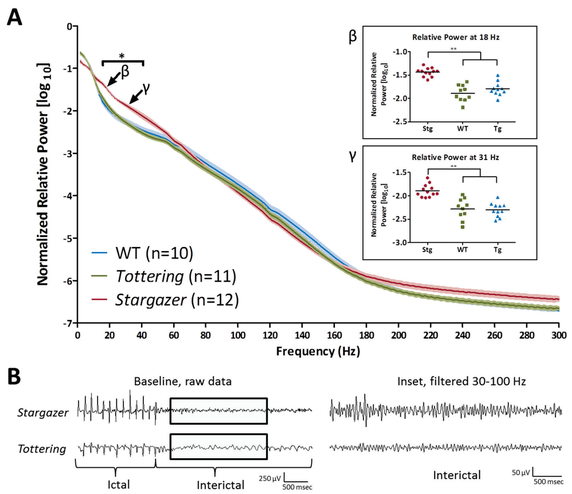
Since PV+ interneurons not only provide a powerful means of inhibiting epileptic seizures, but have also been linked to gamma power in cortical neuronal networks[3], a biomarker for drug efficacy may be hidden at high frequencies in the electroencephalogram (EEG). Given that fast oscillations can be generated by fast-spiking, PV+ interneurons and *stargazer* mice have a mutation which 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 mice at baseline and in response to drugs which have variable effects on seizures.

Methods

Mice were surgically implanted bilaterally with silver wire electrodes inserted into the somatosensory cortex. EEG signals were recorded at 2 kHz and then notch filtered using EEGLab[4] in MATLAB (Mathworks, Inc). Mice acclimated to the recording environment for 30 minutes, video-EEG was then collected for a 30 minute baseline sampling period, followed by intraperitoneal drug injection and the period 30-60 minutes after drug injection was analyzed. All *in vivo* experiments were initiated between 12-2pm.



Seizure activity was defined by spike and wave discharges with an amplitude greater than or equal to 1.5x baseline. Statistical differences pre- and post-injection were tested using a repeated-measures 2-way ANOVA with Bonferroni correction to compare groups over time, with significance set at $p<0.05$. (Prism 5, version 5.0d, GraphPad, CA).



Baseline augmentation in beta/gamma power in *stargazer* mice

(A) Mean +/- standard error of relative power between WT, tottering and *stargazer* from 2-300 Hz. 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).

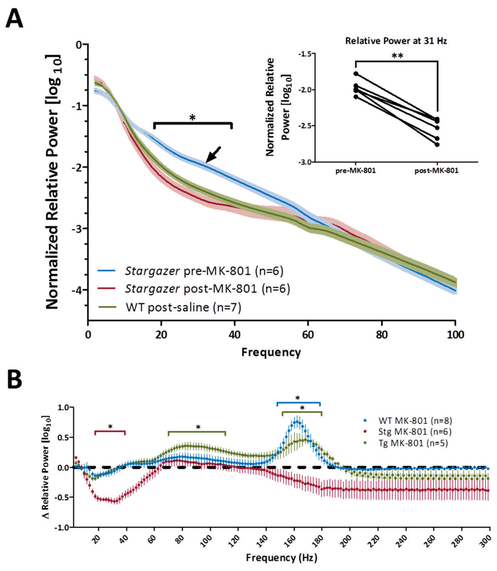
(B) Example of raw interictal EEG sampled at 2 kHz (left) with representative traces filtered between 30-100 Hz (inset, right).

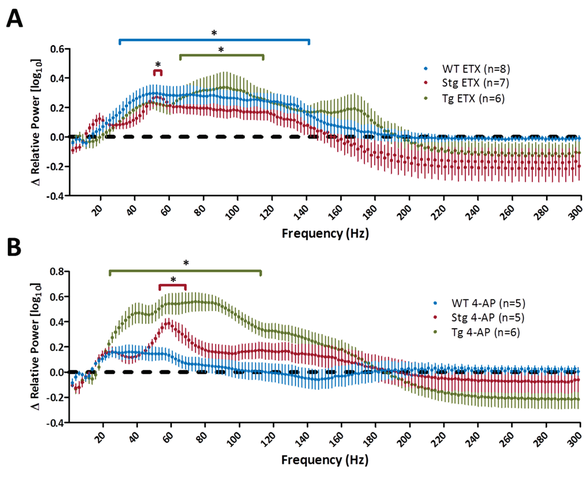
MK-801 has an opposite effect on relative gamma power in *stargazer* and *tottering* mice

As seen previously[2], MK-801, an NMDA receptor antagonist, caused seizure exacerbation with irregular 3-4 Hz spike-wave discharges in stargazer mice (mean ± SEM, 3.46x ± 0.86x relative seizure duration, n=6, p=0.0002).

(A) Recovery of augmented beta/gamma power (18-39 Hz) following 0.5 mg/kg MK-801 injection in *stargazer* mice (mean±SEM, *p<0.05). The maximum difference was at 31 Hz (arrow/inset, **p<0.001).

(B) Elevation in relative power between 148-178 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).



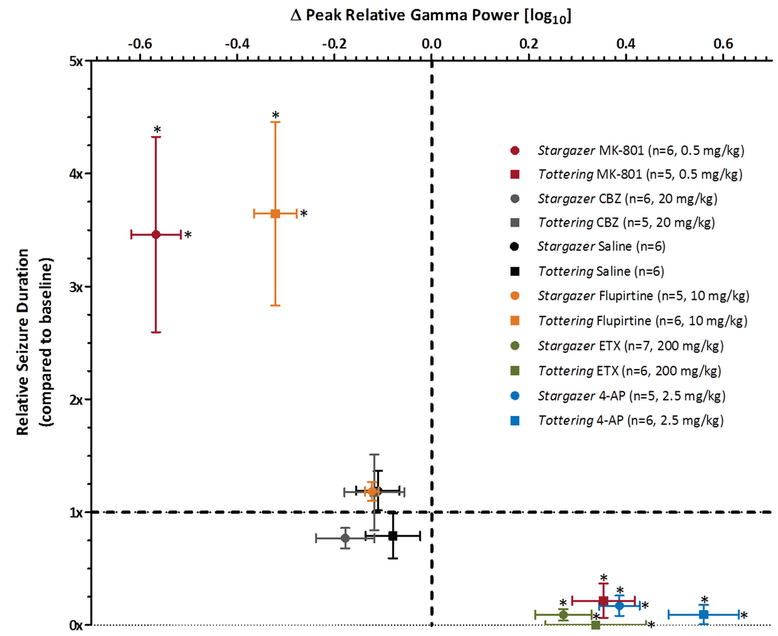


Ethosuximide and 4-aminopyridine significantly reduce seizures and increase relative gamma power

(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).

The shift in peak interictal relative gamma power is inversely correlated with a drug's effect on seizures



Inverse correlation between AED efficacy (mean±SEM) and change in peak relative gamma power (mean±SEM, *p<0.05). Drug responses cluster into 3 groups based on relative seizure duration. Seizure aggravation (top) is associated with reduction in relative gamma power while 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).

Summary

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

References

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Sources of funding

J Noebels: NIH R01 29709; A Maheshwari: Caroline Wiess Law Fund for Molecular Medicine and Department of Neurology, Baylor College of Medicine