Intravenous Administration of Ganaxolone Attenuates Electroencephalographic Seizures in a Diazepam Resistant Model of Status Epilepticus

MARINUS PHARMACEUTICALS

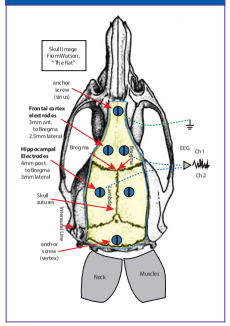
OVERVIEW

- Status epilepticus (SE) is a severe, life-threatening epileptic condition.
- The current treatment paradigm for SE is initial treatment with benzodiazepines to control SE followed by standard antiepileptic drugs such as phenytoin or valproic acid and then administration of general anesthetics such as propofol.
- Patients with SE become progressively refractory to treatment over time. Moreover, more than 30% of SE patients are resistant to aggressive benzodiazepine administration. Thus, treatments that block treatment resistant forms of SE are needed to adequately treat these severe forms of SE.
- Allopregnanolone (ALLO) and ganaxolone (GNX) exhibit anti-convulsant activity in various preclinical models of epilepsy. These compounds elicit their anti-epileptic activity through positive allosteric modulation of both the synaptic and, unlike benzodiazepines, the extrasynaptic gamma-aminobutyric acid ($GABA_A$) receptor.
- Status epilepticus can be modeled in experimental animals by administration of lithium and pilocarpine. Rats subjected to experimental SE are refractory to benzodiazepine if treatment is initiated ≥ 15 min after seizure onset; thus modeling a treatment resistant form of SE.
- The current studies were designed to compare the anti-epileptic activity of GNX and ALLO in this treatment resistant model of SE.

METHODS

- Adult male Sprague-Dawley rats (10 weeks of age) were pre-implanted with cortical electrodes (**Figure 1**).
- Rats were administered lithium chloride (127 mg/kg) followed by pilocarpine (50 mg/kg) to induce SE. Scopolamine was administered prior to pilocarpine to dampen peripherally mediated pilocarpine actions.
- SE was first identified by presence of large amplitude slow wave EEG activity and then behaviorally using the Racine scale. Racine stage 3 seizures were used as the time-marker for SE onset and subsequent treatment.
- GNX, ALLO, or diazepam were administered via intravenous (IV) bolus injection 15 or 60 minutes after detection of SE onset.

Figure 1. Electrode Placement



- Seizure magnitude and duration were measured by EEG recordings. EEG power (mV2/Hz) was calculated by fast Fourier Transform (FFT) in 1 Hz frequency bins from 1 to 96 Hz using ICELUS software. Frequency ranges analyzed:
  - Delta 0 – 5 Hz
  - Theta 5 – 10 Hz
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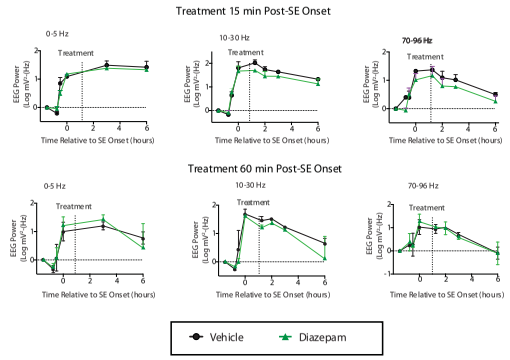
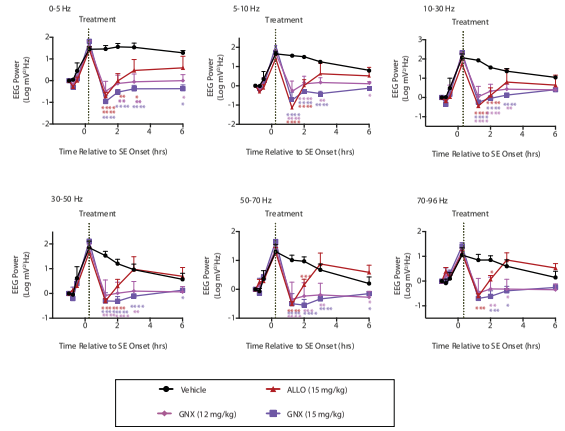
- EEG power values for each animal were normalized by subtracting the baseline value from all subsequent values.
- EEG power data were evaluated by 2-way ANOVA (treatment and time period) at each frequency range using Prism Graphpad (version 6). Post-hoc analysis consisted of Holms-Sidak multiple comparison tests, with significance set at $P < 0.05$.

Figure 2. Experimental Status Epilepticus is Resistant to Diazepam Treatment



Status epilepticus (SE) was induced by administration of lithium and pilocarpine. Diazepam (10 mg/kg; IV) was administered 15 or 60 minutes after SE onset. SE was measured for an additional 5 hrs. Seizures were measured by EEG response. Data were subjected to fast Fourier transformation (FFT) and are expressed as a EEG power (Log mV²/Hz) over pre-determined frequency ranges. Data were analyzed by two-way ANOVA followed by a post-hoc Holms-Sidak test. Diazepam administration did not produce a statistically significant reduction in EEG power.

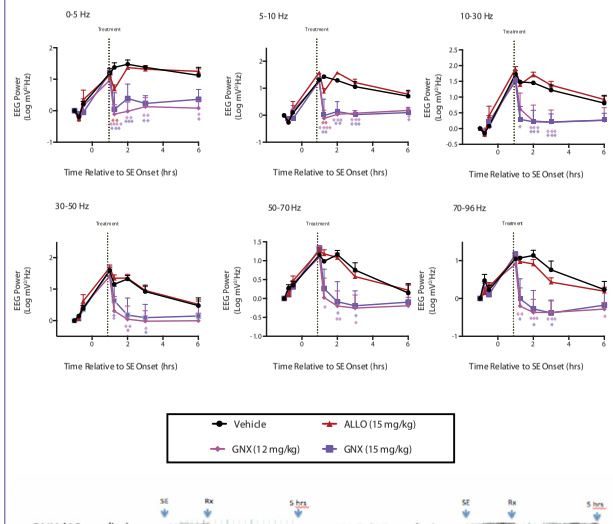
Figure 3. Ganaxolone Elicits a Durable Anti-Epileptic Effect when Administered IV 15 minutes after SE Onset



Ganaxolone (GNX) (12 and 15 mg/kg) or allopregnanolone (ALLO) (15 mg/kg) were administered 15 minutes after status epilepticus (SE) onset and EEG seizure response measured for 5 additional hours. GNX at both dose-levels produced a durable reduction in seizure response (≥ 5 hrs). ALLO produced a shorter duration response (≤ 1 hr).

Statistical analysis: Data analyzed by two-ANOVA followed by post-hoc Dunnett's test. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Figure 4. Ganaxolone Blocks Status Epilepticus when Administered IV 1 hr after SE Onset



GNX (12 and 15 mg/kg) or ALLO (15 mg/kg) were administered 60 minutes after SE onset and EEG seizure response measured for 5 additional hours. GNX at both dose-levels produced a durable reduction in seizure response (≥ 5 hrs). ALLO produced a shorter duration response (≤ 1 hr).

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CONCLUSIONS

- Lithium-pilocarpine administration produces an electrophysiological status epilepticus response.
- This SE is resistant to diazepam administration when initiated 15 or 60 minutes after SE onset.
- GNX administration elicits a sustained (≥ 5 hrs) block of SE independent of treatment time after SE onset.
- In comparison, (ALLO) administration produced only a short term ≈ 1 hr block of SE when administered 15 min after onset, and only a partial block when administered 60 min after onset.
- GNX is also available as an oral formulation allowing the possibility that GNX can be administered orally after SE to prevent subsequent seizure activity.

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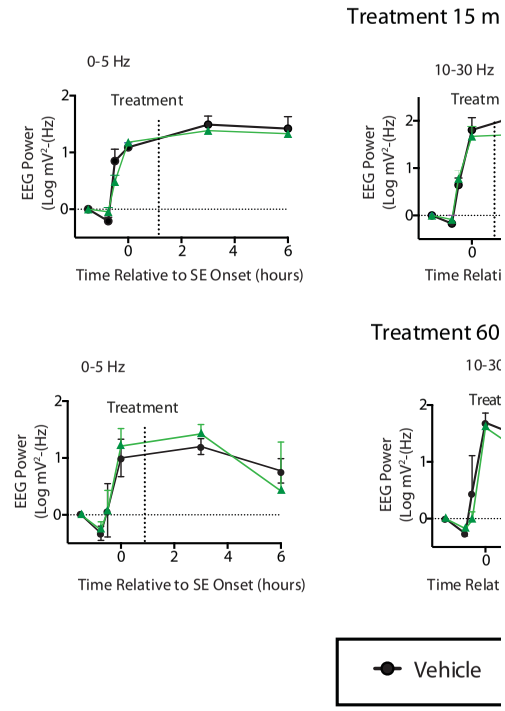

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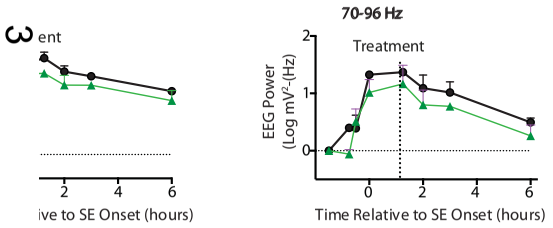

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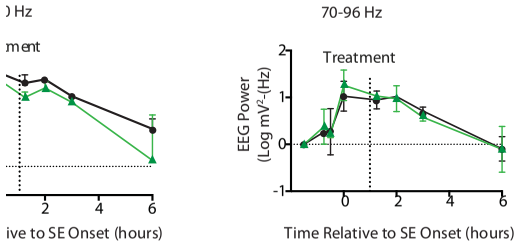

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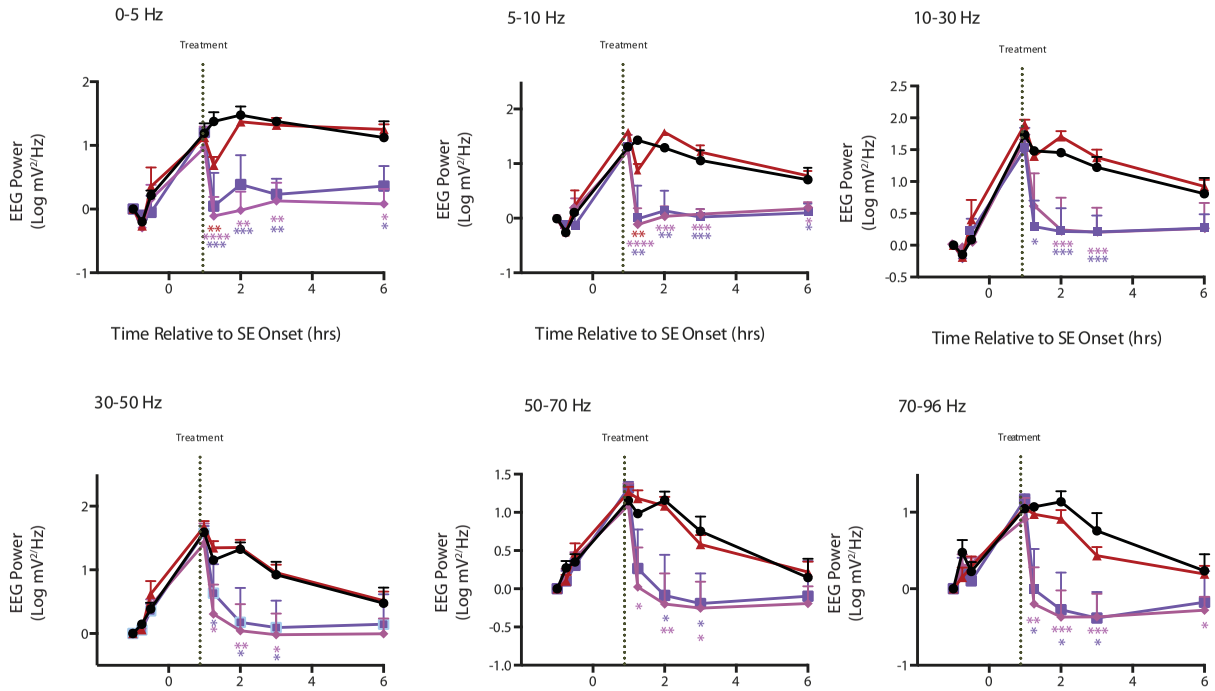

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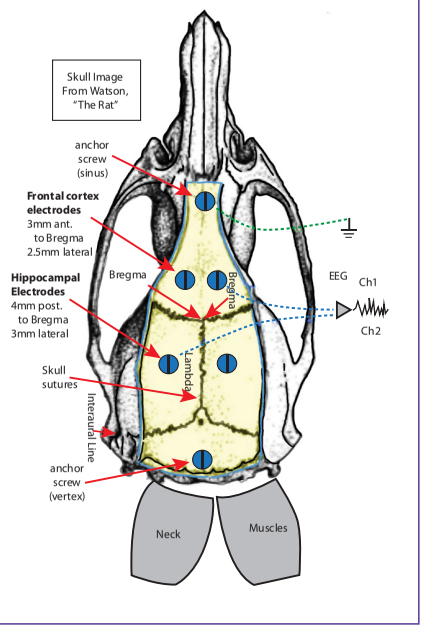

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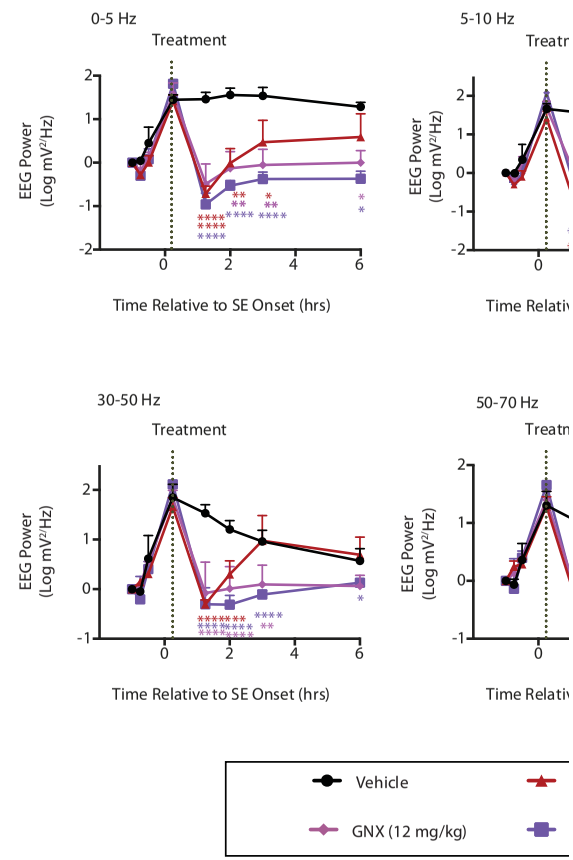

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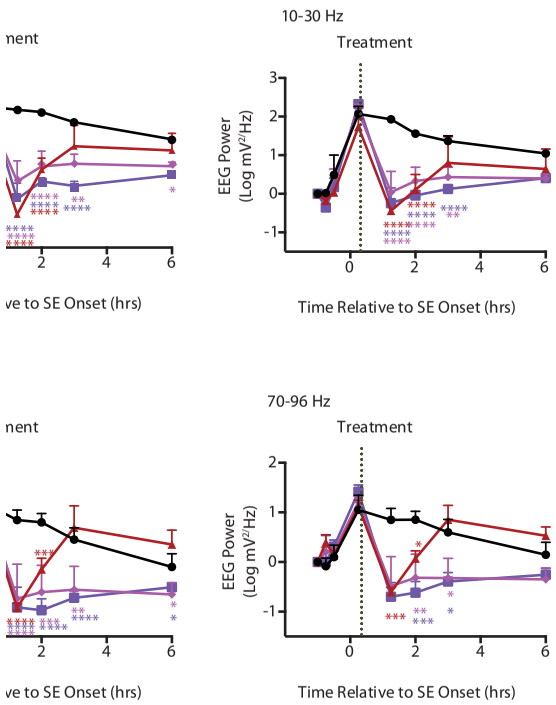

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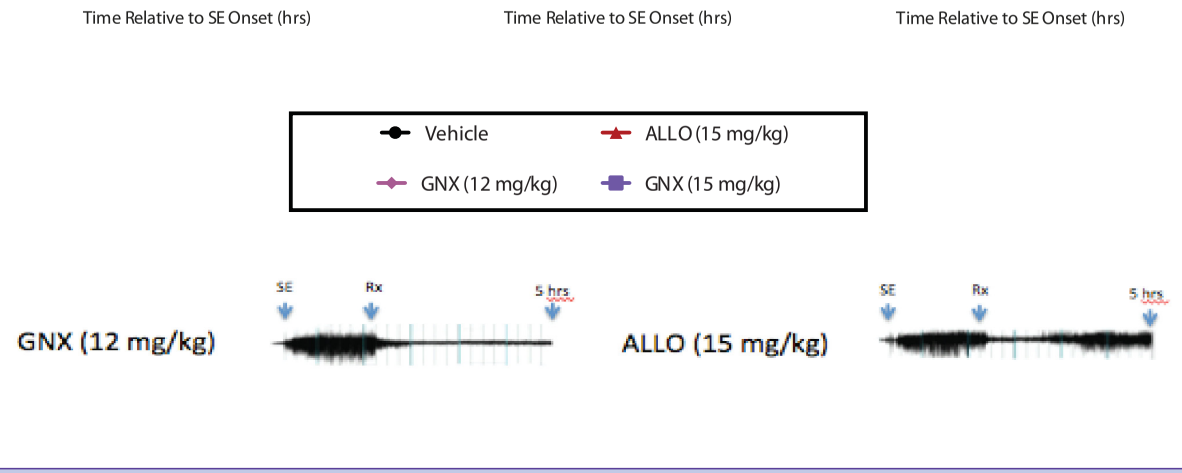

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