

# Translational Pharmacology of PRAX-944, a Novel T-Type Calcium Channel Blocker in Development for the Treatment of Essential Tremor

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**ABSTRACT: Background:** Essential tremor is the most common movement disorder with clear unmet need. Mounting evidence indicates tremor is caused by increased neuronal burst firing and oscillations in cerebello-thalamo-cortical circuitry and may be dependent on T-type calcium channel activity. T-type calcium channels regulate sigma band electroencephalogram (EEG) power during non-rapid eye movement sleep, representing a potential biomarker of channel activity. PRAX-944 is a novel T-type calcium channel blocker in development for essential tremor.

**Objectives:** Using a rat tremor model and sigma-band EEG power, we assessed pharmacodynamically-active doses of PRAX-944 and their translation into clinically tolerated doses in healthy participants, informing dose selection for future efficacy trials.

**Methods:** Harmaline-induced tremor and spontaneous locomotor activity were used to assess PRAX-944 efficacy and tolerability, respectively, in rats. Sigma-power was used as a translational biomarker of T-type calcium channel blockade in rats and, subsequently, in a

phase 1 trial assessing pharmacologic activity and tolerability in healthy participants.

**Results:** In rats, PRAX-944 dose-dependently reduced tremor by 50% and 72% at 1 and 3 mg/kg doses, respectively, without locomotor side effects. These doses also reduced sigma-power by ~30% to 50% in rats. In healthy participants, sigma-power was similarly reduced by 34% to 50% at 10 to 100 mg, with no further reduction at 120 mg. All doses were well tolerated.

**Conclusions:** In rats, PRAX-944 reduced sigma-power at concentrations that reduced tremor without locomotor side effects. In healthy participants, comparable reductions in sigma-power indicate that robust T-type calcium channel blockade was achieved at well-tolerated doses that may hold promise for reducing tremor in patients with essential tremor. © 2022 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society

**Key Words:** essential tremor; T-type calcium channel; EEG; translational biomarkers; sigma power

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Medicines, and is a shareholder. M.W. was an employee of Praxis Precision Medicines during study conduct and manuscript preparation, serves as a paid consultant to Praxis Precision Medicines, and is a shareholder. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this review is consistent with those guidelines.

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Essential tremor (ET) is the most common movement disorder, affecting approximately 7 million individuals in the United States alone.<sup>1</sup> As an action tremor, ET manifests during voluntary movements, interfering with basic functions and causing direct disability<sup>2,3</sup> and impaired job performance.<sup>4</sup> Approximately 50% of patients seek pharmacological therapy; however, almost half discontinue medication due to limited efficacy and poor tolerability.<sup>5,6</sup> Current first-line therapies include propranolol, primidone, and topiramate.<sup>7-10</sup> Deep brain stimulation and/or focused ultrasound thalamotomy are generally effective as last-line options; however, many patients do not choose these procedures due to their invasiveness and risk of irreversible side effects.<sup>5,11,12</sup> A critical unmet need thus remains for patients with ET who are refractory to, or are underserved by, available treatment options.

A large body of preclinical and clinical data demonstrates that aberrant burst firing and oscillatory activity in the cerebello-thalamo-cortical (CTC) circuit are main drivers of tremor.<sup>13,14</sup> The CTC circuit comprises the inferior olivary nucleus, Purkinje cells of the cerebellar cortex, deep cerebellar nuclei, ventral motor thalamus, and motor cortex, which together coordinate movements, and can generate tremor when dysregulated.<sup>15</sup> T-type calcium ( $\text{Ca}^{2+}$ ) channels play a critical role in modulating neuronal firing patterns within the CTC circuit by controlling the switch between tonic and burst firing.<sup>16</sup> T-type  $\text{Ca}^{2+}$  channels are low-voltage-activated channels that respond to weak depolarization of neuronal membranes while in a hyperpolarized state, leading to brief bursts of action potentials, thereby regulating rebound burst firing following inhibitory input.<sup>16</sup> When T-type  $\text{Ca}^{2+}$  channel conductance is increased, either due to genetic mutations or other network activity alterations that increase channel activity, a longer-lasting depolarization is generated, leading to high-frequency bursts of action potentials.<sup>16</sup>

Various lines of evidence point to the importance of T-type  $\text{Ca}^{2+}$  channels as potential therapeutic targets in ET. Whole-exome sequencing of early-onset familial ET has identified mutations in *CACNA1G*, the gene encoding the T-type  $\text{Ca}^{2+}$  channel isoform  $\text{Ca}_v3.1$ , that segregates with tremor phenotype in several family pedigrees.<sup>17</sup> Electrophysiological recordings in patients with ET have demonstrated that activity in multiple CTC nuclei oscillates coherently with tremor in affected limbs.<sup>18,19</sup> Furthermore, intraoperative single neuron recordings in the ventral motor thalamus of patients with severe ET have revealed strong temporal association between tremor and burst firing, while reduced burst firing through deep brain stimulation in this region directly correlates with tremor reduction.<sup>20,21</sup> Due to their role in the CTC circuit, T-type  $\text{Ca}^{2+}$  channel blockade and subsequent normalization of burst firing in the CTC network may therefore provide effective treatment in ET.<sup>22</sup>

In addition to their relevance in ET, T-type  $\text{Ca}^{2+}$  channels play important roles in regulating electroencephalographic (EEG) patterns during sleep. Specifically, EEG power in the sigma frequency band (11–15 Hz,  $\sigma$ -power) is prominent during non-rapid eye movement (NREM) sleep.<sup>23,24</sup> Although published data on the effects of selective T-type  $\text{Ca}^{2+}$  channel blockers on NREM  $\sigma$ -power are lacking, NREM  $\sigma$ -power has been shown to be reduced in mice lacking T-type  $\text{Ca}^{2+}$  channels,<sup>25-27</sup> an effect mediated, at least in part, by reduced burst firing of cortically-projecting thalamic neurons.<sup>27</sup> As such, we hypothesized that NREM  $\sigma$ -power may represent a mechanistically relevant and translational central pharmacodynamic biomarker of T-type  $\text{Ca}^{2+}$  channel blockade.

PRAX-944 (formerly Z944) is a potent and selective small molecule T-type  $\text{Ca}^{2+}$  channel blocker.<sup>28</sup> In the current study, we first sought to determine brain and plasma concentrations associated with PRAX-944 doses that robustly reduce tremor in the rat harmaline model without locomotor side effects. We further investigated whether these doses would be associated with changes in NREM  $\sigma$ -power as a biomarker of T-type  $\text{Ca}^{2+}$  channel blockade. We subsequently used NREM  $\sigma$ -power as a translational biomarker in a Phase 1 trial of healthy participants to determine whether similarly pharmacodynamically-active doses of PRAX-944 are well tolerated, with the objective of informing selection of potentially efficacious doses for future ET trials.

## Methods

### Preclinical

All studies involving use of animals were reviewed by local institutional ethics committees and performed in accordance with national guidelines for the ethical use of animals for research.

### *In Vivo* Pharmacology

#### *Rat Harmaline-Induced Tremor*

PRAX-944 was profiled in the rat harmaline-induced tremor model across three studies (see Appendix S1). Briefly, rats were administered PRAX-944 (0.1–30 mg/kg, orally [PO]) or vehicle (0.5% methyl cellulose, 0.1% Tween80 in water) 60 minutes prior to harmaline injection. Rats were placed in a recording chamber fitted with a piezoelectric recording plate for a 20-minute baseline recording. Rats were then dosed with harmaline (30 mg/kg, intraperitoneally [IP]) and returned to the chamber for 30 minutes, with 10 to 30 minutes post-harmaline considered the test period.

To measure harmaline-induced tremor, the piezoelectric signal was processed using a fast Fourier transform (FFT), with a frequency range of 8 to 12 Hz determined as the harmaline-induced tremor band.<sup>29</sup> Tremor power

fold-change was calculated as average tremor power test/baseline. For each experiment, plasma and brain tissue samples were collected from a subset of rats and PRAX-944 concentrations measured.

### **Rat Spontaneous Locomotor Activity**

The spontaneous locomotor activity (sLMA) test was used to evaluate tolerability and potential motor side effects of PRAX-944 (see Appendix S1). Male Sprague Dawley rats were administered either PRAX-944 (3–300 mg/kg, PO) or vehicle (0.5% methyl cellulose, 0.1% Tween80 in water). Sixty minutes post-dose, rats were visually assessed for signs of gross motor impairment, including ataxia and sedation, using a four-point scoring system (Table S1) and placed into an open field chamber to measure distance traveled over a 30-minute observation period. Plasma and brain tissue samples were collected from a subset of animals for PRAX-944 concentration quantification.

### **Rat In Vivo EEG**

The effects of PRAX-944 on quantitative EEG (qEEG) were assessed via electrocorticography in rats (see Appendix S1). Briefly, adult male Sprague Dawley rats were implanted with skull screws and neck electromyography (EMG) electrodes connected to telemetry devices. EEG was performed at least 7 days post-surgery after full recovery. PRAX-944 effects were assessed in two crossover studies, each with a minimum 3-day washout between sessions. EEG was continuously recorded from 2 hours prior to treatment (baseline period), through 12 hours following PRAX-944 (1–100 mg/kg, PO) or vehicle (0.5% methyl cellulose, 0.1% Tween80 in water) administration (test period). Raw EEG recordings were manually scored to identify sleep stages in 10-second epochs and spectral analysis performed using FFT on each epoch; epochs were averaged within each sleep stage in 30-minute bins for the first 4 hours post-dose, and in 60-minute bins for subsequent timepoints. For each sleep stage, total power in each test period time bin was determined relative to the sleep scored 2-hour baseline period. Plasma and brain tissue samples were collected from satellite animals 1-hour post-dose to measure PRAX-944 concentrations.

### **Statistical Analysis**

Statistical analyses were conducted using GraphPad Prism 7.0. In vivo data were assessed for normality using the Shapiro–Wilk test. Normally distributed data were analyzed using a one-way analysis of variance (ANOVA) for comparison of PRAX-944-treated groups to vehicle, and corrected using Dunnett’s test. Non-normally distributed data were analyzed using the

Kruskal–Wallis test, with two-tailed Mann–Whitney tests used for pairwise group comparison of treatment versus vehicle, corrected using Dunn’s test. EEG data were analyzed by ANOVA followed by Tukey’s post hoc tests. Dose–response and concentration–response curves for plasma and brain were fitted for each endpoint.

### **Clinical**

PRAX-944-105 was a two-part, Phase 1 clinical trial of a fixed dose titration of PRAX-944 conducted in healthy participants (18–55 years) to assess safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) parameters utilizing qEEG and polysomnography (PSG) ([anzctr.org](http://anzctr.org); ACTRN12620000675921). The trial was conducted in Melbourne, Australia (May 7 to September 6, 2020) and approved by the local ethics committee. Informed consent was given by all screened participants. Sample sizes were selected to provide sufficient safety, PK, and PD data to inform future trials.

### **Study Design**

Part A was open-label and assessed PK and PD qEEG/PSG effects of a fixed oral dose titration regimen of 5, 10, and 20 mg of PRAX-944 over 12 days in a single cohort (N = 8 subjects). Each dose level was sequentially administered once per morning (qAM) for 4 days. Twenty-four-hour qEEG/PSG assessments were performed at baseline (day –1) and at steady-state after dosing 5, 10, and 20 mg PRAX-944 (days 4, 8, and 12, respectively).

Part B was randomized, double-blind, placebo-controlled, and assessed PK and PD qEEG/PSG effects of a different fixed titration regimen of PRAX-944. Enrolled participants were randomized to receive PRAX-944 or placebo in a 3:1 ratio (PRAX-944 N = 12, placebo N = 4). Participants randomized to PRAX-944 completed 3 days of dosing at 20 mg qAM (days 1–3) and 3 days at 40 mg qAM (days 4–6). On day 7, participants began 60 mg qAM, at which point dosing continued for 7 days before escalating to the next level. This scheme was repeated at each dose level until reaching 120 mg. Dose titration allowed for 4 days at each level to achieve steady-state plasma concentrations and obtain qEEG/PSG assessments, plus an additional 3 days to allow for collection of PK and safety data to guide safety review committee determination of dose escalation. Twenty-four-hour qEEG/PSG assessments were performed at Baseline (day –1) and at steady state after dosing 60, 80, 100, and 120 mg (days 10, 17, 24, and 31, respectively). Plasma PK samples were collected and assayed by liquid chromatography–tandem mass spectrometry.

### Clinical EEG Recordings and Polysomnography

Twenty-four-hour EEG recordings were performed with a standard 10 to 20 montage with additional chin electrodes for collecting EMG data used for sleep analysis. Two electrooculogram leads were used to remove eye movement artifacts. Raw EEG signals (sampled at 200 Hz) were recorded by referencing each channel to the Oz electrode, then re-referenced to averaged mastoid (M1 and M2) electrodes. EEG recordings were inspected visually to reject segments contaminated by high-frequency noise artifact.

Sleep stages were classified as REM or NREM stages 1, 2, or 3 (N1, N2, N3, respectively) by an automated sleep scoring algorithm (ASEEGA software, Physip, Paris, France).<sup>30</sup> Stages N1–3 were merged and referred to here as NREM sleep. Using 512-point segments of artifact-free data, the power spectrum was computed with a Hanning window and FFT independently for each sleep stage. For each subject, median power spectra were calculated in 30-second epochs of NREM sleep and averaged across the whole session. Power spectra were then averaged across the central nine electrodes (ie, F3, Fz, F4, C3, Cz, C4, P3, Pz, P4) and group means calculated across subjects for each dose and timepoint to generate group spectrograms. Absolute NREM  $\sigma$ -power was calculated as the area under the curve between 11 and 15 Hz. Topographic maps of NREM  $\sigma$ -power were generated by averaging absolute NREM  $\sigma$ -power for each electrode across subjects, dose, and timepoint. Change from baseline was defined as the ratio of absolute NREM  $\sigma$ -power at timepoint to baseline; with baseline values defined as those from the last EEG assessment prior to the first treatment day (ie, day –1). For graphical purposes, results are presented as percent change from baseline for each treatment day, based on median values ( $\pm$ interquartile range).

### Safety Assessment

Safety endpoints included physical examination, clinical and laboratory evaluations, vital sign assessment, 12-lead ECG, cardiac telemetry, the Columbia Suicide Severity Rating Scale (C-SSRS), and adverse event monitoring (Table S2).

### Statistical Analysis

Statistical analyses were conducted using SAS v9.4 and GraphPad Prism 7.0. For Part B, absolute NREM  $\sigma$ -power values for each electrode on each day of testing were natural-log (ln) transformed to conform with normality assumptions and averaged across the central nine electrodes. A mixed model repeated measures (MMRM) method was used for analysis

of group differences in NREM  $\sigma$ -power change from baseline, which included timepoint, treatment (PRAX-944 or placebo), and treatment-by-timepoint interaction as fixed factors, and baseline value as a covariate. Model-based point estimates (ie, least square [LS] mean change for each treatment group and the difference between treatment groups), 95% confidence interval (95% CI) for the difference, and *P* values are reported for each assessment timepoint.

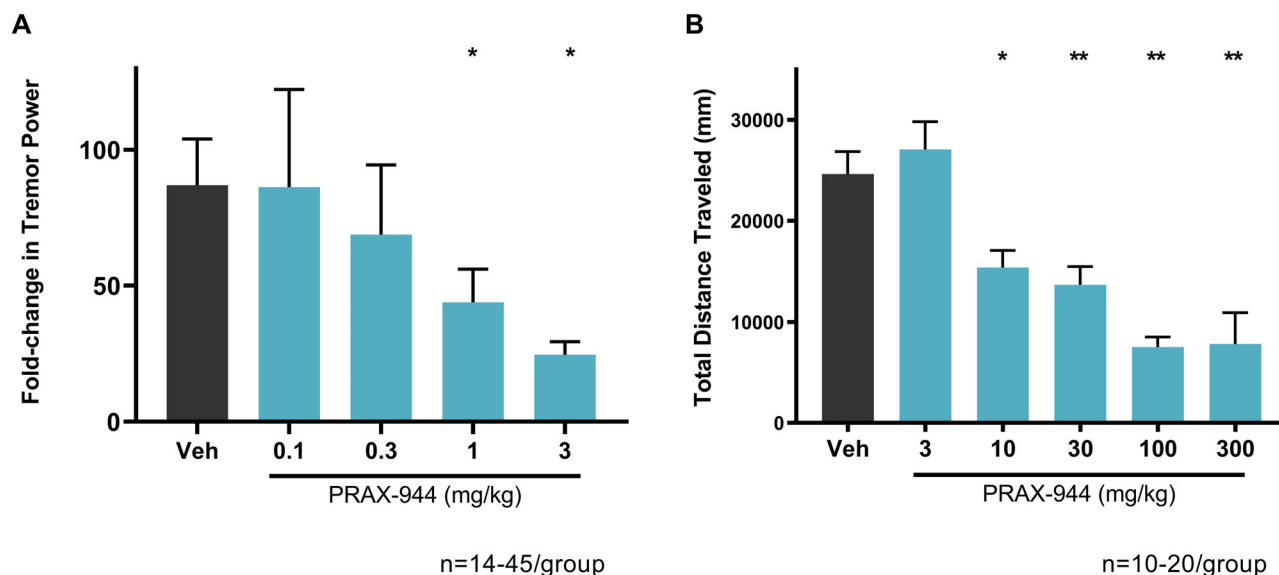
## Results

### PRAX-944 Reduces Harmaline-Induced Tremor in Rats

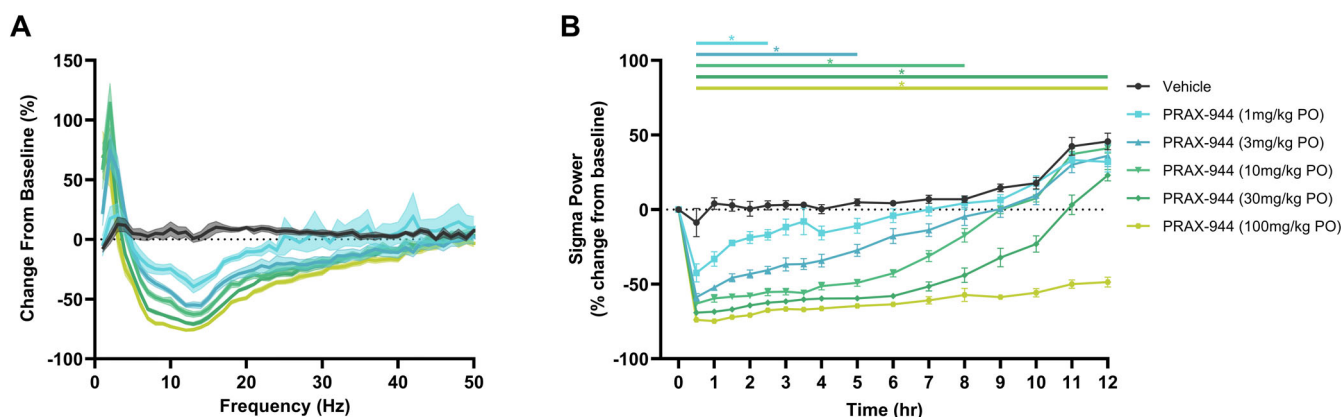
Studies have shown that the harmaline-induced tremor model is sensitive to systemic administration of T-type  $\text{Ca}^{2+}$  channel blockers.<sup>31,32</sup> Harmaline (30 mg/kg, IP) produced a robust induction of tremor power (8–13 Hz). Only data from the 0.1 to 3 mg/kg dose groups were included for harmaline-induced tremor analysis due to spontaneous locomotor suppression observed with higher doses (10–30 mg/kg; see sLMA below). Pretreatment with PRAX-944 (0.1–3 mg/kg, PO) dose-dependently reduced harmaline-induced tremor power (Fig. 1A). A 50% reduction in tremor power was observed following treatment with 1 mg/kg PRAX-944; associated mean plasma and brain concentrations were 43 ng/mL and 68.9 ng/g, respectively. A greater tremor reduction (72%) was observed in the 3 mg/kg dose group, with mean plasma and brain concentrations of 121 ng/mL and 173 ng/g, respectively. Associated dose and concentration response curves are shown in Figure S1.

### PRAX-944 Only Reduces Activity in the Rat sLMA Test at Higher Concentrations

Since compounds that reduce harmaline-induced tremor may do so by non-specifically suppressing locomotor activity, PRAX-944 tolerability and potential motor side effects were assessed using the sLMA test. While PRAX-944 at the 3 mg/kg dose showed activity similar to that of vehicle, 10 to 300 mg/kg doses significantly reduced total distance moved in the 30-minute sLMA test (Fig. 1B). The calculated dose required to produce 50% reduction in locomotor activity ( $\text{TD}_{50}$ ) was 45.6 mg/kg and the calculated drug concentrations in plasma and brain associated with 50% response ( $\text{TC}_{50}$ ) were 5538 ng/mL and 3172 ng/g, respectively (Figure S2). No rats receiving PRAX-944 up to 100 mg/kg exhibited gross motor impairments, including sedation or ataxia, as scored via visual assessment. Of the 10 rats dosed with PRAX-944 300 mg/kg, 7 exhibited mild motor impairment, and 1 exhibited moderate effects.



**FIG. 1.** PRAX-944 reduces (A) harmaline-induced tremor and (B) distance traveled over 30 minutes in the spontaneous locomotor activity test in rats. Fold-change in tremor power = average power post-harmaline/average baseline power (pre-harmaline). Mean ± SEM displayed. \**P* < 0.05, \*\**P* < 0.01 versus vehicle (Veh). SEM, standard error of the mean.



**FIG. 2.** PRAX-944 (1–100 mg/kg) reduces non-rapid eye movement (NREM)  $\sigma$ -power in rats. (A) Change in NREM spectral power relative to baseline, by treatment group (30–60 minutes after treatment). (B) Time course of NREM  $\sigma$ -power change by treatment group (over 12 hours). Treatment administered at time = 0 hour. Data based on *n* = 11 per group and presented as percent change from baseline for recordings from the frontal/posterior cortex. Spectral power is presented in 30-minute bins for 0 to 4 hours post-dose and 60-minute bins for 4 to 12 hours. Mean ± SEM displayed. \**P* < 0.05 versus vehicle. SEM, standard error of the mean; PO, orally.

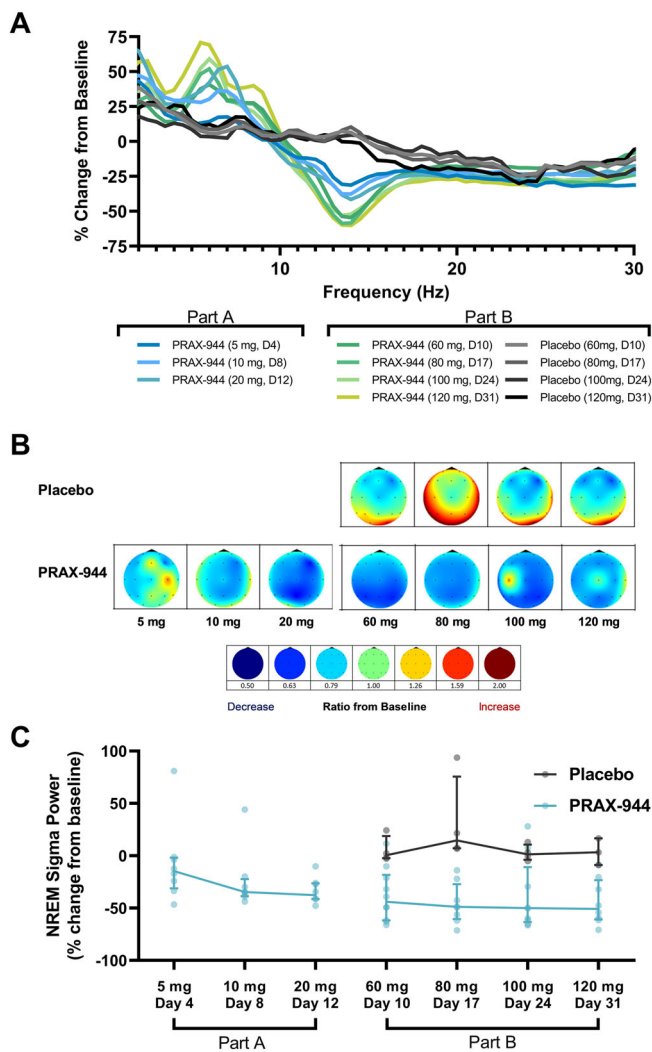
### PRAX-944 Reduces NREM $\sigma$ -Power and Other Frequency Bands in Rats

The effects of PRAX-944 on qEEG were assessed in freely moving rats, using NREM  $\sigma$ -power as a pharmacodynamic biomarker of T-type  $\text{Ca}^{2+}$  channel blockade. The most notable effects of PRAX-944 on EEG occurred during NREM sleep. All dose levels (1–100 mg/kg) had robust, dose-dependent effects on spectral power during NREM sleep (Fig. 2A). Delta power was increased, while theta, alpha, sigma, and beta powers were decreased. The decrease in  $\sigma$ -power began within 30 minutes of dosing and was maintained in periods of NREM sleep across the entire 12 hours of recording following administration of the highest dose

of PRAX-944 (100 mg/kg) (Fig. 2B). NREM  $\sigma$ -power was reduced by 33% following administration of 1 mg/kg PRAX-944, with mean plasma and brain concentrations of 52.9 ng/mL and 57.5 ng/g, respectively. The 3 mg/kg dose reduced NREM  $\sigma$ -power by 52% and was associated with plasma and brain concentrations of 115.5 ng/mL and 125.6 ng/g, respectively. Associated dose and concentration response curves are shown in Figure S3.

### PRAX-944 Reduces NREM $\sigma$ -Power in Healthy Participants

Given the robust reduction of NREM  $\sigma$ -power in rodents at brain and plasma concentrations associated



**FIG. 3.** PRAX-944 reduces non-rapid eye movement (NREM)  $\sigma$ -power in healthy participants (PRAX-944-105). **(A)** Percentage change in absolute NREM spectral power relative to baseline by treatment group. Data are presented in 0.5 Hz frequency bins. **(B)** Electroencephalogram (EEG) flat maps depicting absolute NREM  $\sigma$ -power change from baseline across each electrode. Data are presented per group as mean ratios of absolute NREM  $\sigma$ -power to baseline. **(C)** Absolute NREM  $\sigma$ -power change from baseline. Data presented as percent change from baseline based on the average of the nine central electrodes. Circles denote values from individual participants, lines represent median  $\pm$  interquartile range. D = day; NREM, non-rapid eye movement.

with significant tremor reduction in the rat harmaline model, we investigated whether we could achieve similar pharmacodynamic effects in healthy participants in a two-part Phase 1 clinical trial (Figure S4). Participant baseline characteristics are shown in Table S3. As hypothesized, robust dose-dependent reductions in NREM  $\sigma$ -power were observed across the 24-fold dose range tested over the 31-day treatment period (5–120 mg, Fig. 3A). Reductions in NREM  $\sigma$ -power were consistently observed across multiple electrodes at each dose tested (Fig. 3B). Furthermore, NREM  $\sigma$ -power was reduced by approximately 34% to 49% at 10 to 80 mg, with a maximum

reduction of 50% achieved at 100 mg (Fig. 3C), comparable to reductions observed preclinically. Spectral analysis of sleep-scored EEG data during NREM sleep also revealed increased theta power (4–8 Hz); however, this was variable and did not appear to be dose-dependent (Fig. 3A). MMRM analysis (Part B) demonstrated significant reductions in NREM  $\sigma$ -power relative to placebo, with comparable group differences observed across all PRAX-944 doses (60–120 mg; Table 1).

## PRAX-944 Was Well Tolerated in Healthy Participants

PRAX-944 was well tolerated up to 120 mg daily with no serious or severe adverse events (AEs). Most AEs were mild and resolved without intervention (Table S4). In Part A, the most frequently reported treatment emergent adverse events (TEAEs) were dry eye, headache, and medical device site dermatitis (37.5%). In Part B, the most frequently reported TEAEs were medical device site dermatitis (PRAX-944 25%; placebo 50%), headache (PRAX-944 16.7%; placebo 50%), and dizziness (PRAX-944 25%; placebo 0%). No treatment-related ECG or EEG abnormalities were observed at any dose. Aside from a slight reduction in time spent in NREM sleep at higher doses (Part B), there were no apparent treatment-related changes in other sleep parameters (Figure S5). No vital signs or physical examination findings were reported as AEs. Safety laboratory values were generally within normal limits, with no dose-dependent excursions. No treatment-emergent changes in the Columbia-Suicide Severity Rating Scale (C-SSRS) were observed. A maximum tolerated dose level was not determined for PRAX-944 in this trial.

## Discussion

Dysregulated burst firing and abnormal oscillations in the CTC circuit have been implicated as drivers of ET, with an established role for their regulation by T-type  $\text{Ca}^{2+}$  channels.<sup>16</sup> Here, we have demonstrated that PRAX-944, a novel T-type  $\text{Ca}^{2+}$  channel blocker, dose-dependently reduced harmaline-induced tremor in rats at doses that did not impair normal motor function. In addition, PRAX-944 dose-dependently reduced NREM  $\sigma$ -power in both rodents and healthy participants, representing a potential translational biomarker of central T-type  $\text{Ca}^{2+}$  channel blockade.

NREM  $\sigma$ -power is thought to be driven, in part, by brief bursts of oscillatory activity in the 11 to 15 Hz range (ie, sleep spindles), which are known to be dependent on T-type  $\text{Ca}^{2+}$  channel activity in thalamo-cortical circuitry.<sup>33</sup> Thus, our preclinical and clinical findings of reduced NREM  $\sigma$ -power indicate that PRAX-944 reached target exposures needed to block T-type  $\text{Ca}^{2+}$  channels, suggesting a link between pharmacodynamically-active

**TABLE 1** Mixed model repeated measures analysis of non-rapid eye movement (NREM)  $\sigma$ -power change from baseline by treatment group (PRAX-944-105, Part B)

Timepoint, dose	Placebo (N = 4)		PRAX- 944 (N = 10)		PRAX-944 vs. Placebo		P value
	LS means	SEM	LS means	SEM	Difference (95% CI)	Difference ratio (95% CI)	
Day 10, 60 mg	0.055	0.171	-0.542	0.108	-0.597 (-1.042, -0.152)	0.550 (0.353, 0.859)	0.0132
Day 17, 80 mg	0.260	0.253	-0.437	0.163	-0.697 (-1.360, -0.035)	0.498 (0.257, 0.965)	0.0407
Day 24, 100 mg	0.031	0.137	-0.698	0.094	-0.729 (-1.094, -0.364)	0.482 (0.335, 0.695)	0.0011
Day 31, 120 mg	0.123	0.183	-0.556	0.118	-0.679 (-1.158, -0.200)	0.507 (0.314, 0.819)	0.0098

Note: Change from baseline in NREM  $\sigma$ -power (ln-transformed values) analyzed using a mixed model repeated measures model with an unstructured covariance pattern and fixed effects of timepoint, treatment group, and treatment-by-timepoint interaction and covariate of baseline value.

Note: Difference ratio estimates for the comparison between treatment and placebo were calculated as the exponentiated LS mean difference [exp (Difference)].

Abbreviations: LS, least squares; SEM, standard error of the mean; CI, confidence interval.

and efficacious doses. As such, we postulate that NREM  $\sigma$ -power reduction can be used as a translational pharmacodynamic biomarker of T-type  $\text{Ca}^{2+}$  channel blockade and guide dose selection for future efficacy trials in patients with ET. In our preclinical studies, a 33% to 52% reduction in NREM  $\sigma$ -power was observed at doses with corresponding plasma and brain concentrations that reduced harmaline-induced tremor by 50% and 72%, respectively. In humans, comparable NREM  $\sigma$ -power reductions of 34% to 50% in the 10 to 100 mg dose range indicate robust T-type  $\text{Ca}^{2+}$  channel blockade, suggesting these doses may hold therapeutic promise for reducing tremor in patients with ET. Notably, NREM  $\sigma$ -power effects were maintained, and PRAX-944 well-tolerated, at doses up to 120 mg. Furthermore, NREM  $\sigma$ -power reduction throughout the 31-day dosing period suggests PRAX-944 can achieve sustained T-type  $\text{Ca}^{2+}$  channel blockade.

Consistent with published data implicating T-type  $\text{Ca}^{2+}$  channel in harmaline-induced tremor,<sup>3</sup> our preclinical work demonstrated that PRAX-944 dose-dependently reduced harmaline-induced tremor in rats. Notably, tremor was reduced at doses devoid of signs typically indicative of broader motor suppression. The harmaline-induced tremor model has long been used to assess preclinical efficacy of ET treatments in rodents.<sup>29,34</sup> Harmaline, an alkaloid toxin, induces an acute tremor and rhythmic burst-firing activity in the CTC circuit resembling the increased burst firing observed in patients with ET.<sup>34</sup> It increases synchrony and oscillatory activity of inferior olive neurons,<sup>19,35</sup> leading to excitatory climbing fiber responses and resultant complex firing in Purkinje cells, as well as subsequent burst firing in deep cerebellar nuclei and the ventral motor thalamus.<sup>35,36</sup> Normalizing oscillatory activity in the CTC circuit in rodents (eg, via deep brain stimulation) reduces harmaline-induced tremor.<sup>37</sup> Similarly, compounds that reduce tremor in patients (eg,

propranolol, ethanol) reduce harmaline-induced tremor preclinically,<sup>29</sup> whereas compounds shown to worsen tremor (eg, caffeine) also worsen harmaline-induced tremor.<sup>38</sup> Notably, an absence of subthreshold oscillations and rhythmic burst firing in the inferior olive has been shown in  $\text{Ca}_v3.1$  knockout mice, resulting in resistance to harmaline-induced tremor, while selective  $\text{Ca}_v3.1$  knockdown via shRNA infused directly into the inferior olive reduces harmaline-induced tremor in wild-type mice.<sup>31</sup> Our results therefore suggest a role for PRAX-944 in tremor reduction via modulation of T-type  $\text{Ca}^{2+}$  channel blockade within the CTC circuit.

The harmaline-induced tremor model has previously only demonstrated 56% concordance with efficacy in human trials, with sensitivity to agents such as apomorphine, carbamazepine, and valproate that do not reduce tremor in patients with ET observed preclinically.<sup>39,40</sup> This is likely due to these treatments reducing overall motor activity (eg, sedation or ataxia), and limiting the ability to initiate movement, rather than specifically reducing tremor.<sup>41</sup> Given the associated potential for false-positives, we conducted independent behavioral tolerability tests, demonstrating that while PRAX-944 produced dose-dependent reductions in rat sLMA, significant motor side effects were only observed at doses higher than those associated with tremor reduction (1 and 3 mg/kg). As such, we hypothesize that PRAX-944 exerts a specific effect on reducing tremor at lower doses via T-type  $\text{Ca}^{2+}$  channel blockade within the CTC circuit, with non-specific locomotor effects only evident at supratherapeutic doses. Ongoing Phase 2 trials in patients with ET will provide added insight into these proposed relationships (NCT05021978/NCT05021991, [ClinicalTrials.gov](https://clinicaltrials.gov)); the first, an open-label trial, provides preliminary support for this hypothesis, with tremor reduction observed at well-tolerated PRAX-944 doses.<sup>42</sup> The second, a randomized double-blind, placebo-controlled, dose-range

finding trial of PRAX-944, will further inform clinically efficacious and well-tolerated doses for later-phase efficacy trials.

Given studies in T-type  $\text{Ca}^{2+}$  channel knockout mice have demonstrated that NREM  $\sigma$ -power and underlying sleep spindles are dependent on T-type  $\text{Ca}^{2+}$  channel activation,<sup>25-27</sup> it is plausible that the reduced NREM  $\sigma$ -power observed with PRAX-944 reflects a reduction in sleep spindles. Of note, rodent and human studies suggest that sleep spindles contribute to processes related to memory consolidation during sleep.<sup>43</sup> The existing preclinical literature on the effects of T-type  $\text{Ca}^{2+}$  channel blockers on learning and memory, however, are mixed, reporting either impaired or enhanced mnemonic function depending on the behavioral assay and experimental model used.<sup>44,45</sup> Furthermore, these studies have only tested acute effects of T-type  $\text{Ca}^{2+}$  channel blockade, and therefore do not address potential effects on mnemonic functions theorized to be dependent on sleep spindles. Although the sample size in our clinical trial was small, and memory functions were not specifically tested, there were no reports of impaired memory over the 31-day treatment period.

We have demonstrated that administration of PRAX-944 in rats and humans produced strong and consistent effects on NREM  $\sigma$ -power, representing a potential translational biomarker of T-type  $\text{Ca}^{2+}$  channel blockade. Furthermore, in rats, reduced NREM  $\sigma$ -power was observed at PRAX-944 doses, and corresponding brain and plasma concentrations, that reduced harmaline-induced tremor. In humans, comparable NREM  $\sigma$ -power reductions suggest that we achieved similar T-type  $\text{Ca}^{2+}$  channel blockade at well-tolerated doses. Importantly, our clinical and preclinical findings suggest that the wide dose range of PRAX-944 tested in healthy participants may hold promise for reducing tremor in patients with ET. Ongoing clinical investigations of PRAX-944 will provide valuable further insights into the therapeutic potential of T-type  $\text{Ca}^{2+}$  channel blockers in patients with ET. ■

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### Data Availability Statement

The data that support the findings reported here are available from the corresponding author, LS, upon reasonable request.

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## Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.