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Mechanisms of tremor-modulating effects of primidone and propranolol in essential tremor

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A B S T R A C T

Introduction: Primidone and propranolol are primary treatments for essential tremor, however the exact mechanisms underlying their efficacy are not fully elucidated. Understanding how these medications alleviate tremor may guide the development of additional pharmacologic treatments. Our prospective observational study employed transcranial magnetic stimulation (TMS) to explore mechanisms of primidone and propranolol effects in essential tremor. Eyeblink classical conditioning (EBCC) was tested as a potential predictor of treatment response.

Methods: Patients with essential tremor underwent two evaluations: prior to commencing primidone or propranolol and following a minimum of three months of treatment. Tremor severity was assessed using accelerometry and clinically. TMS was employed to study changes in corticospinal excitability - resting and active motor thresholds, resting and active input/output curves and intracortical excitability - cortical silent period (CSP), short interval intracortical inhibition intensity curve (SICI), long interval intracortical inhibition (LICI), intracortical facilitation (ICF), and short afferent inhibition (SAI). EBCC, a marker of cerebellar function, was studied at baseline.

Results: Of the 54 enrolled patients (28 primidone, 26 propranolol), 35 completed both visits. Primidone effect on decreasing hand tremor was associated with decreased corticospinal excitability, prolongation of CSP, increased LICI, increased SAI and decreased SICI. Propranolol effect on hand tremor was associated with decreased corticospinal excitability and increased SAI. Better EBCC at baseline predicted better response to primidone.

Conclusions: Primidone exerts its therapeutic effects by blocking voltage-gated sodium channels and by modulating GABA-A and GABA-B intracortical circuits. Propranolol's central effects are likely mediated via noradrenergic modulation of GABA outflow.

1. Introduction

Essential tremor (ET) is the most common movement disorder. Its pathophysiology is not fully understood, which poses challenges for the development of novel symptomatic medications [1,2]. Primidone and propranolol are first-line therapies for ET and the sole medications for treating ET with the level A recommendations according to the latest guidelines of the American Academy of Neurology [3]. The discovery of their effectiveness in ET was serendipitous and the precise mechanisms responsible for their tremor-improving effects remain unclear [4–8]. Understanding these mechanisms can guide the development of additional symptomatic treatments.

After oral intake, primidone is partially metabolized to phenylethylmalonamide that has no anti-tremor activity and phenobarbital, that improves tremor, likely through positive modulation effect on GABA-A receptors [9–12]. Through an unknown mechanism, unmetabolized primidone also demonstrates anti-tremor activity, as evidenced by its higher effectiveness compared to phenobarbital alone and its faster impact on tremor relative to the formation of its metabolites [7,13,14]. While there is no dispute that propranolol acts peripherally, by blocking beta-2 adrenergic receptors in muscle spindles, its effect on tremor via central beta-adrenergic receptors is uncertain [6,7].

Transcranial magnetic stimulation (TMS) can activate targeted neuronal circuits in the human brain, allowing in vivo assessment of drug effects on neurotransmitter systems that impacts these circuits. In particular, motor evoked potentials (MEPs) produced at motor thresholds are believed to occur through monosynaptic connections between the superficial excitatory pyramidal neurons and large pyramidal tract neurons and drugs that act on voltage-gated sodium channels have been shown to increase motor threshold [15,16]. In contrast, MEPs generated at higher TMS intensities arise from a more complex network of excitatory circuits modulated by inhibitory circuits [16,17]. Short-interval intracortical inhibition (SICI) is thought to reflect GABA-A mediated inhibition, while long-interval intracortical inhibition (LICI) and cortical

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silent period (CSP) reflect GABA-B mediated inhibition. Short-latency afferent inhibition (SAI) reflects the activity of cholinergic circuits, which are modulated by GABAergic circuits [18]. A set of well-defined TMS measures can therefore be employed to examine the pattern of effects associated with drugs possessing unknown or multiple modes of action [15].

In this prospective observational study, patients with ET intended to start treatment with either primidone or propranolol, underwent two separate sessions, comprising of clinical, accelerometric and TMS evaluations, with the objective of understanding the mechanisms contributing to tremor alleviation. We additionally applied eyeblink classical conditioning (EBCC) protocol to assess if conditioning deficit, as a marker of cerebellar impairment in ET [19,20], may predict future response to symptomatic treatment.

2. Materials and methods

2.1. Participants

Fifty-four individuals diagnosed with ET and previously untreated for tremor were included from a movement disorders outpatient clinic. ET diagnosis followed the latest consensus statement on tremor classification. Presence of plus features, i.e. "ET plus" was allowed [21]. The choice of medication (primidone or propranolol) was made at the discretion of the treating neurologist, who also provided instructions for dose escalation. Exclusion criteria were use of medications, aside from primidone and propranolol, that could impact TMS measurements.

2.1.1. Study design

Patients underwent two experimental sessions: (1) before initiating primidone or propranolol (baseline) and (2) after at least 3 months (range 3–6 months) of treatment. In both sessions tremor severity was assessed using The Essential Tremor Rating Assessment Scale - Performance (TETRAS-P) [22] (hand tremor severity was sum of items 4, 6, 7, and 8), patient-rated visual analogue scale (VAS 0–10) and accelerometry. Patients also underwent a brief clinical examination to assess the presence of rest tremor, intention tremor, any signs of head or arm dystonia and tandem gait assessment. Baseline session also included demographic data collection and EBCC paradigm. TMS was performed in both sessions (at the same time of day for each participant), with second TMS performed 2–4 h after last propranolol and approximately 12 h after last primidone dose.

Due to the logarithmic relationship between tremor amplitude (T) and perceived severity (clinical scores R), the equation $T2\text{-}T1/T1 = 10^{\alpha(R2-R1)}-1$ was used to estimate percentage change.[23] Here, $\alpha = 0.03125$ for the total TETRAS-P score and $\alpha = 0.0455$ for the hand tremor sub-score) [24].

2.2. TMS measures

TMS was performed over the primary motor cortex contralateral to the most affected hand in the case of asymmetric tremor (as revealed by the TETRAS assessment), or over the dominant hemisphere (determined by the reported writing hand preference), in the case of symmetric tremor. Electromyography (EMG) recordings were made from the abductor pollicis brevis muscle with Ag-AgCl surface electrodes using a belly-tendon montage. Single- and paired-pulse TMS of the primary motor cortex was applied using Magstim 200 magnetic stimulators with a monophasic current waveform (Magstim Company, Whitland, UK) connected to a standard figure-of-eight coil. Hotspot point, resting motor threshold (RMT), active motor threshold (AMT), resting and active input/output (IO) curves, cortical silent period (CSP) curve, short-interval intracortical inhibition curve (SICI curve), intra-cortical facilitation (ICF) and long-interval intracortical inhibition (LICI) were performed as described previously [25]. For short afferent inhibition (SAI), the median nerve was stimulated at the wrist with a standard bar electrode using a constant current stimulator (Digitimer model DS7A; Digitimer), with conditioning stimulus (pulse width 200 μs) intensity adjusted just over motor threshold for evoking a visible twitch of the thenar muscles and test stimulus adjusted to 120 % of RMT and inter-stimulus interval (ISI) 20, 22 and 24 ms. TMS data were analyzed by a blinded investigator. For details on TMS measure acquisition see Supplementary appendix.

2.3. Eye blink classical conditioning (EBCC)

Conditioning stimulus (CS) was a loud (70–80 dB; 2000 Hz) tone lasting 400 ms, delivered via binaural headphones. The unconditioned stimulus (US) was an electrical pulse of 200 μs length and of intensity equal to five times the sensory threshold, delivered over the right supraorbital nerve 400 ms after the CS. Surface EMG was recorded bilaterally from the orbicularis oculi muscle. The test consisted of seven blocks. The first six blocks consisted of 9 CS-US pairs, 1 US only, 1 CS only trial and the seventh block consisted of 11 CS-only trials. There was a random interval (from 10s to 30s) between CS-US pairs to minimize habituation [26].

2.4. Accelerometry

A triaxial accelerometer (Biometrics Ltd; sensitivity ±50 mV/G) was attached on the third metacarpal bone 1 cm proximal to the metacarpophalangeal joint, bilaterally. Accelerometry was recorded under three conditions for at least 30 s (but no longer than 45 s), while subjects were seated in an armchair with elbows and forearms supported: (a) hands pronated and completely relaxed (resting position) (b) hands outstretched at wrist (postural condition) and (c) hands outstretched with a 500 g mass attached to the hand (weight loading in postural condition).

2.4.1. Signal analysis

Accelerometry data were captured using Spike 2 version 6 (Cambridge Electronic Design).

Tremor signals were bandpass filtered between 2 and 30 Hz [27]. Postural recordings were analyzed in all patients to determine tremor frequency and amplitude, to assess the effects of primidone and propranolol. Additionally, recordings during weight loading were analyzed in all patients to demonstrate that weight loading does not influence tremor frequency, thereby confirming the central component of the tremor. Recordings during the rest condition were analyzed in patients who clinically exhibited rest tremor, providing data on rest tremor amplitude that was included in the main analysis to control for possible confounding effects of rest tremor on TMS measures. Composite data, obtained by calculating the square root of the sum of the acceleration squared for all three axes, were analyzed [27–29]. The initial 30 s for each condition were analyzed. These epochs were windowed using a Hanning window, and a Fast Fourier Transformation (FFT) was applied to each epoch using a sliding window approach. The tremor frequency was identified by detecting the frequency of the first dominant peak in the spectrum [30]. The root mean square (RMS) amplitude was calculated from the total power within the 1–30 Hz range [31]. Additionally, the amplitude at the peak frequency was determined by taking the square root of the power of the main peak [31]. This dual analysis provides a comprehensive assessment of both the overall tremor severity and the intensity at the primary tremor frequency.

2.5. Data preparation

For the analysis of TMS parameters the Boltzmann three-parameter sigmoidal function was fit to the IO curves and CSP curve, and maximum MEP value (MEP max), peak slope (PS), and area under the fitted curve (AUC) were calculated [32,33].

2.6. Statistics

IBM SPSS Statistics Version 22 and RStudio software were used for the analyses. Demographic and clinical data between the primidone and propranolol groups were compared using Chi-square tests for categorical variables and independent t-tests (parametric or non-parametric as appropriate) for continuous variables. Separate Generalized Linear Mixed Models (GLMMs) with linear or gamma distributions assessed intervention effects (primidone or propranolol) on tremor severity (clinical scores, accelerometry) and TMS parameters. Models explored differences between the effects of primidone and propranolol by including time ("no treatment" vs. "on treatment") × treatment type ("primidone" vs. "propranolol") interaction term. Models assessing tremor severity controlled for age, gender, disease duration, and the presence of ET-plus signs, while models for TMS measures additionally controlled whether the dominant or non-dominant hemisphere was stimulated and for the RMS amplitude of the rest tremor before and after treatment. Random effects used individual intercepts. SICI and SAI analyses included stimulus intensity (4 levels: 70–100 % RMT) and interstimulus intervals (3 levels: ISI: 20 ms, 22 ms, 24 ms), respectively, including time × treatment type interaction. Accelerometry data from both hands were included in the analysis of treatment effects. All available data were included in the GLMM analysis, ensuring that data from patients with only one visit were also analyzed. Post hoc tests were conducted when the main effect of time or the time × treatment type interaction was significant. Specifically, we computed the estimated marginal means (EMMs) for TMS measures over time, separately for primidone and propranolol and conducted contrasts between these EMMs, employing the Holm-Bonferroni correction to adjust for multiple comparisons. To ensure a comprehensive assessment of drug effects, we calculated contrast estimates and standardized effect sizes (Cohen's d) for all outcomes, including non-significant results using the "emmeans" package in R [34]. This approach helps to avoid the common pitfall of overlooking potentially meaningful trends that may not have reached statistical significance due to inadequate sample size. Additionally, we conducted a post-hoc power analysis, with a detailed description and the results provided in the Supplementary Appendix.

Repeated measures correlations were used to assess the within-subject relationship between TMS measures and tremor amplitude, before and after treatment. Correlations were calculated using the "rmcorr" package in R [35,36], focusing on TMS measures that showed a significant treatment effect. For details, see the Supplementary Appendix.

An additional GLMM assessed whether baseline EBCC predicted treatment response, with change in TETRAS tremor severity as outcome and average EBCC, treatment type (propranolol or primidone), the interaction between treatment type and EBCC, age, sex and disease duration as predictors. Random effects used individual intercepts. We investigated the interaction between EBCC and treatment type by estimating separate slopes for each treatment group.

When necessary, nonparametric data were logarithmically transformed for normality.

All tests were two-tailed with the significance level set at $p \leq 0.05$.

3. Results

3.1. Patients: clinical and demographic data

54 patients were included in the study; of these 28 were prescribed primidone (15 men, 13 women, median age 72.5 years {IQR: 65.25–76.0}, median disease duration 3.75 years {IQR: 2.5–16.5}) and 26 propranolol (13 men, 13 women, median age 70.0 years {IQR: 46.0–74.25}, median disease duration 9.5 years {IQR: 2.75–30.5}). Twenty-eight patients exhibited additional signs (ET-plus), including rest tremor (N = 12), intention tremor (N = 16), impaired tandem gait (N = 4), and questionable dystonia (N = 2). Rest tremor was of lower amplitude than action tremor in all affected patients, with 5 showing unilateral and 7 showing bilateral rest tremor. Of two patients with questionable dystonia, one had mild torticollis and one possible dystonic finger posturing. Median daily dose of primidone and propranolol was 250 mg (IQR: 203–250 mg) and 80 mg (IQR: 75–80 mg), respectively. There were no statistically significant differences in demographic and clinical data between the primidone and propranolol groups. Detailed demographic and clinical data, along with statistical comparisons between the groups are presented in Table 1. Nineteen patients withdrew after the initial assessment: 8 from the primidone group and 11 from the propranolol group. Of these, 11 (5 from primidone and 6 from propranolol group) discontinued treatment due to side effects, while 8 were unwilling to attend the second visit.

3.2. Treatment effect - clinical data

For the TETRAS-P hand tremor sub-score, there was a significant effect of time ($F(1, 29) = 15.02$, $p = 0.001$). Post-hoc analysis revealed that both primidone (contrast estimate (CE): −2.61, 95 % CI: −4.75 to −0.48, $p = 0.018$) and propranolol (CE: −4.35, 95 % CI: −6.79 to −1.91, $p = 0.001$) decreased hand tremor (Fig. 1). For the total TETRAS-P score, there was also a significant effect of time ($F(1, 46) = 13.90$, $p = 0.001$), however post-hoc analysis revealed that only propranolol significantly decreased total TETRAS-P score (CE: −5.57, 95 % CI: −8.83 to −2.30, $p = 0.001$). For the tremor severity assessed on VAS, there was also a significant effect of time ($F(1, 27) = 15.10$, $p = 0.001$), with post-hoc revealing that both primidone (CE: −12.95, 95 % CI: −23.39 to −2.52, $p = 0.017$) and propranolol (CE: −17.932, 95 % CI: −30.42 to −5.44, $p = 0.006$) decreased subjective tremor rating. For details see Supplementary Table 1 in Supplementary appendix.

3.3. Treatment effect - accelerometry data

For accelerometry-measured RMS tremor amplitude and amplitude at PF, there was a significant effect of time ($F(1,76) = 21.44$, $p < 0.001$ and $F(1,95) = 54.42$, $p < 0.001$, respectively).The interaction treatment *time was not significant. Post hoc tests confirmed that both primidone (CE: −0.058, 95 % CI: −0.105 to −0.012, $p = 0.014$ for RMS amplitude; CE: −0.034, 95 % CI: −0.065 to −0.003, $p = 0.034$ for amplitude at PF) and propranolol (CE: −0.107, 95 % CI: −0.171 to −0.042, $p = 0.001$ for RMS amplitude; CE: −0.051, 95 % CI: −0.098 to −0.004, $p = 0.032$ for amplitude at PF) decreased hand tremor (Fig. 1). Tremor improvement exceeded 25 %, which is a natural variability of ET amplitude reported in the literature [37], in 10 patients (50 %) in primidone and in 8 patients (53 %) in propranolol group. Tremor frequency did not change after loading, as expected for central tremors [38. For details see Supplementary Table 1 in Supplementary appendix.

3.4. TMS measures

Of the 54 patients included, 39 exhibited slightly asymmetric tremor, with 22 of them having the most affected non-dominant hand. Therefore, the dominant hemisphere was stimulated in 32 patients, while the non-dominant hemisphere was stimulated in 22 patients.

3.4.1. Corticospinal excitability: RMT, AMT, rest, and active IO curves (supplementary table 2)

For RMT, there was a significant effect of time ($F(1,31) = 7.74$, $p = 0.009$) and interaction time x treatment type ($F(1,31) = 5.66$, $p = 0.0024$). Post hoc revealed that only primidone increased RMT (CE: 4.60, 95 % CI: 1.99 to 7.22, $p = 0.001$), while propranolol did not affect RMT (Fig. 2). While AMT was slightly higher on treatment compared to baseline, the effect of time or interaction time x treatment type was not significant (Fig. 2).

As for the parameters of the rest IO curve, the effect of time was significant for PS ($F(1,33) = 11.95$, $p = 0.002$) and AUC ($F(1,32) =$

Table 1
Clinical and demographic data.

		Primidone group		Propranolol group	Statistics	p
Males/females		15/13		13/13	χ^2 (1) = 0.069	0.793
Age (Mdn[a], IQR[b])		72.5, 65.25–76.0		70, 46.0–74.25	Z = −1.490	0.136
TETRAS-P [c]						
	Total (M[d] ± SD[e])	22.4 ± 5.5		22.2 ± 8.1	t (52) = 0.125	0.901
	Hand (M ± SD)	19.1 ± 4.3		18.5 ± 6.7	t (52) = 0.375	0.709
	Voice (M ± SD)	1.1 ± 1.0		1.2 ± 0.9	t (52) = −0.199	0.843
	Head (M ± SD)	0.5 ± 0.6		0.4 ± 0.8	t (52) = 0.609	0.545
	Face (M ± SD)	0.5 ± 0.8		0.7 ± 0.9	t (52) = −0.690	0.493
	Lower limb (M ± SD)	0.9 ± 1.1		1.2 ± 1.0	t (52) = −0.940	0.352
	Standing tremor (M ± SD)	0.3 ± 0.8		0.3 ± 0.6	t (52) = 0.072	0.943
VAS[f] score (M ± SD)		4.3 ± 2.6		4.0 ± 3.6	t (52) = 0.277	0.783
Tremor frequency posture (M ± SD)		6.6 ± 0.9 Hz		6.8 ± 0.9 Hz		
Tremor frequency weight (M ± SD)		6.9 ± 1.2 Hz		6.9 ± 1.2 Hz		
RMS[g] tremor amplitude posture (Mdn, IQR)		WH	0.35 mV, 0.18–0.52	0.24 mV, 0.12–0.84	Z = −0.787	0.431
		BH	0.17 mV, 0.14–0.22	0.17 mV, 0.10–0.35	Z = −0.557	0.578
Tremor amplitude at PP[h] posture (Mdn, IQR)		WH	0.20 mV, 0.10–0.42	0.16 mV, 0.09–0.62	Z = −0.517	0.605
		BH	0.10 mV, 0.07–0.18	0.15 mV, 0.06–0.25	Z = −0.742	0.458
Dosage (Mdn, IQR)		250 mg, 250-250		80 mg, 50-80		
ET plus	No.	18		10	χ^2 (1) = 3.601	0.058
	Rest tremor (No.)	8		4	χ^2 (1) = 1.356	0.244
	Rest tremor square root TP (Mdn, IQR)	WH	0.10 mV, 0.09–0.20	0.12 mV, 0.07–0.33	Z = −0.081	0.935
		BH	0.08 mV, 0.07–0.14	0.08 mV, 0.07–0.13	Z = −0.365	0.792
	Intention tremor (No.)	11		5	χ^2 (1) = 0.2601	0.107
	Impaired tandem gait (No.)	3		1	χ^2 (1) = 0.927	0.336
	Dystonia (No.)	1		1	χ^2 (1) = 0.003	0.957
Disease duration (years) (Mdn, IQR)		3.75, 2.5–16.5		19.5, 2.75–30.5	Z = −0.938	0.348
Time between visits (days) (Mdn, IQR)		135.0, 105.8–179.8		101.0, 94.0–181.0	Z = −1.067	0.298
Asymmetric tremor (No.)		22		17	χ^2 (1) = 1.169	0.280
Nondominant hand stimulation (No.)		11		11	χ^2 (1) = 0.013	0.908

[a] Mdn: median.
[b] IQR: interquartile range.
[c] TETRAS-P: The Essential Tremor Rating Assessment Scale–Performance.
[d] M: mean.
[e] SD: standard deviation.
[f] VAS: Visual Analogue Scale.
[g] RMS – rooth mean sqaure of total power (surogate measure for total tremor amplitude).
[h] PP – peak power (surogate measure for peak amplitude).

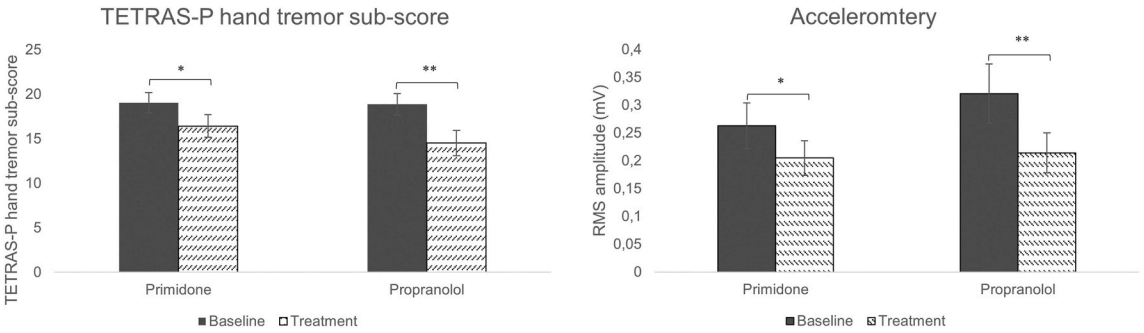

Fig. 1. Treatment effects. The impact of primidone and propranolol on The Essential Tremor Rating Assessment Scale – Performance (TETRAS-P) hand tremor sub-score (hand tremor severity was sum of items 4, 6, 7, and 8) and on postural tremor amplitude, as assessed by accelerometry. Estimated marginal means with standard errors are represented. RMA – root mean square. A single asterisk denotes a significance of $p < 0.05$ and a double asterisk of $p < 0.01$.

26.23, $p < 0.001$), while time x treatment type interaction was significant for MEP max (F (1,28) = 4.96, p = 0.035). Post-hoc analysis showed that both primidone (CE: −49.28, 95 % CI: −72.08 to −26.47, $p < 0.001$) and propranolol (CE: −25.15, 95 % CI: −50.73 to −4.80, p = 0.030) decreased AUC of the resting IO curve (Fig. 2). In addition, primidone decreased MEP max (CE: −1.56, 95%CI: −2.45 to −0.67, p = 0.001) and decreased PS (CE: −0.051, 95 % CI: −0.082 to −0.020, p = 0.002) (Fig. 2).

As for the parameters of the active IO curve, there was a significant interaction time x treatment type for PS (F (1,29) = 4.82, p = 0.036). Post hoc analysis showed that primidone decreased PS of active IO curve (CE: −0.054, 95%CI: −0.092 to −0.017, p = 0.005), while propranolol did not affect it (Fig. 2). No other significant effects of time or time x treatment type interaction were found for other parameters of the active IO curve.

3.4.2. Intracortical inhibition and facilitation: CSP, LICI, SICI, and ICF (Supplementary Table 3)

For the CSP, there was a significant interaction time x treatment type for the area under the CSP curve (F (1,32) = 9.61, p = 0.004). Post hoc analysis showed that primidone significantly increased CSP (CE: 1551 95 % CI:366 to 2737, p = 0.012), while propranolol had no effect (Fig. 3). For LICI, there was a significant interaction time x treatment type (F (1,40) = 5.39, p = 0.025). Post hoc showed that primidone

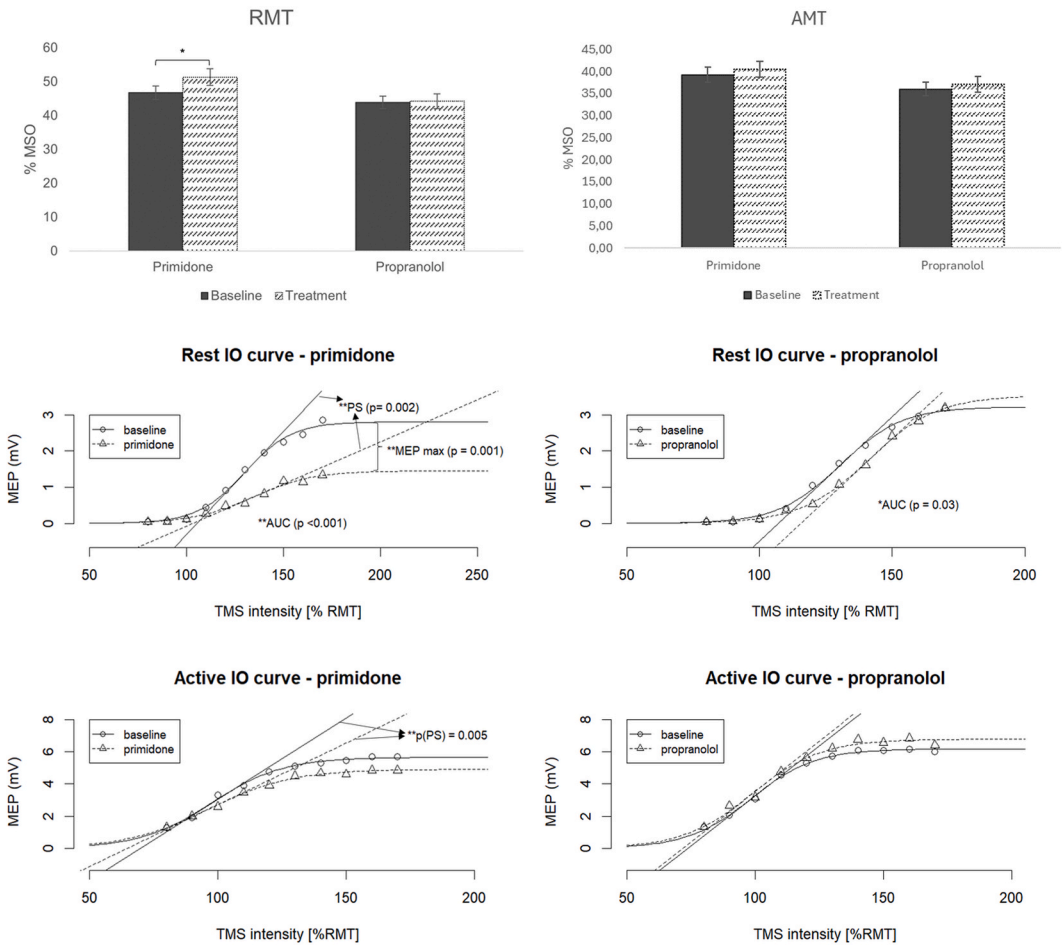

Fig. 2. Effects of primidone and propranolol on resting motor threshold (RMT), active motor threshold (AMT) and on resting and active input/output (IO) curves. MEP max – maximal motor evoked potential; PS – peak slope; AUC – area under the curve; %MSO - the percentage of maximum stimulator output. A single asterisk denotes a significance of $p < 0.05$ and a double asterisk of $p < 0.01$.

enhanced LICI (CE: −0.136, 95%CI: −0.244 to −0.029, p = 0.014), while propranolol had no effect (Fig. 3).

For SICI curve, there was significant interaction time x treatment type (F (1,264) = 14.04, p < 0.001). Post-hoc showed that primidone decreased SICI (CE: 0.20, 95%CI: 0.10 to 0.31, p < 0.001), while propranolol had no effect (Fig. 3).

For ICF, there was no significant effect of time or time x treatment type interaction.

3.4.3. *Short-afferent inhibition (supplementary table 4)*

For SAI, there was significant effect of time (F (1, 183) = 14.85, p < 0.001). Post hoc showed that primidone enhanced SAI at all ISI (CE for 20 ms: −0.20 {95 % CI: −0.37 to −0.04}, p = 0.019; CE for 22 ms: −0.164 {95%CI: −0.32 to −0.01}, p = 0.037, CE for 24 ms: −0.194 {95%CI: −0.35 to −0.04}, p = 0.014), while propranolol increased SAI for ISI of 20 ms only (CE: 0.22, {95%CI: −0.40 to −0.05}, p = 0.015) (Fig. 3).

EMMs before and after treatment, contrast estimates and standardized effect sizes (Cohen's d) for all outcomes, including non-significant results, with corresponding 95 % CI are provided in Table 2. Raw pre-treatment and post-treatment TMS data values are provided in Supplementary Table 5.

3.5. *EBCC*

Median average percentage of conditioned responses was 22.4 % (IQR: 6.9–40.0 %) in primidone and 22.3 % (IQR: 12.1–37.9 %) in propranolol group.

Electrophysiological correlates of primidone and propranolol effects on tremor amplitude (Supplementary Table 6).

In the primidone group, significant repeated measures correlations were found between tremor amplitude, as assessed by accelerometry, and following TMS measures: RMT (r = −0.52, p = 0.022), MEP max (r = 0.51, p = 0.027) and AUC (r = 0.51, p = 0.026) of the rest IO curve, PS of the active IO curve (r = 0.60, p = 0.014), area under the CSP curve (r = −0.516, p = 0.024) and SAI at 22 ms (r = 0.50, p = 0.036). This indicates that, within subjects, increases in RMT, decrease in MEP max and AUC of the rest IO curve, decrease in PS of the active IO curve, increase in the area under the CSP curve and enforcement of SAI were correlated with decreases in tremor amplitude with treatment.

In the propranolol group, significant repeated measures correlations were found between tremor amplitude and AUC of the rest IO curve (r = 0.52, p = 0.039) and between tremor amplitude and SAI at 20 ms (r = 0.69, p = 0.003). This means that within subjects, decrease of AUC of the rest IO curve and enforcement of SAI were correlated with decreases in tremor amplitude with treatment.

3.6. *EBCC as a predictor of response to treatment*

GLMM for improvement in TETRAS-P score with treatment showed significant interaction between EBCC and treatment type (F (1,28) = 6.62, p = 0.016). Separate slope analysis revealed that better EBCC was

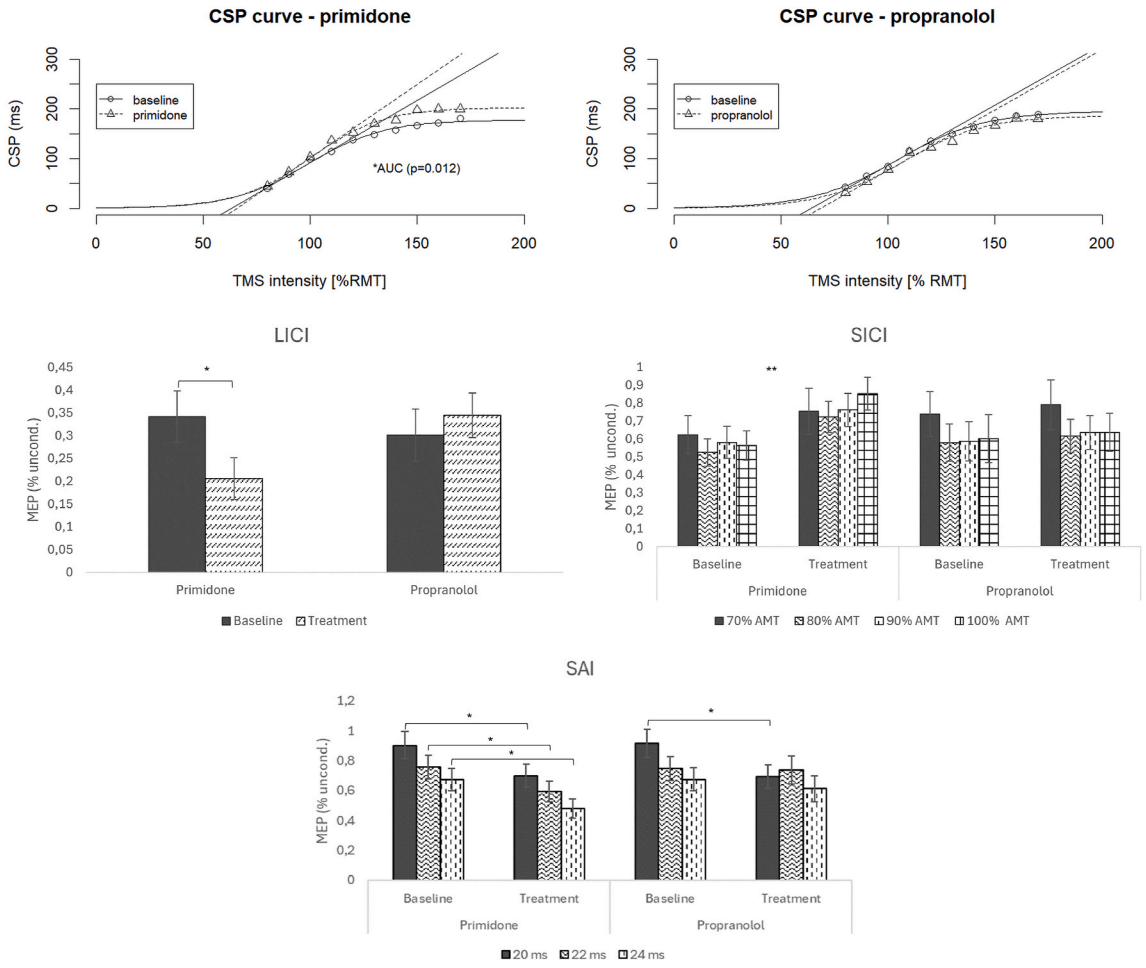

Fig. 3. Effects of primidone and propranolol on transcranial magnetic stimulation measures of intracortical inhibition and on short afferent inhibition (SAI). Estimated marginal means with standard errors are represented. CSP – cortical silent period curve; PS – peak slope; LICI – long intracortical inhibition; SICI - short intracortical inhibition; ISI - interstimulus intervals. A single asterisk denotes a significance of $p < 0.05$ and a double asterisk of $p < 0.01$.

significant predictor of TETRAS-P score improvement on primidone (Exp (b) = 0.238, {95 % CI 0.087 to 0.650}, p = 0.007), while it did not predict improvement on propranolol (Exp (b) = 2.113, {95 % CI 0.626 to 7.139}, p = 0.218).

4. Discussion

Primidone and propranolol were both effective in reducing hand tremor severity in patients with essential tremor (ET), as measured by objective TETRAS and subjective VAS clinical scales, consistent with hand tremor being the primary concern in ET patients [39]. As expected, both medications also decreased postural tremor amplitude, as measured by accelerometry. The response rate on primidone (50 %) and propranolol (53 %) in our study is consistent with previous reports [7,39]. Eighteen percent of patients in primidone and 23 % in propranolol group discontinued treatment, which is a dropout rate similar to previous studies [7,39,40]. Considering mechanisms of anti-tremor effectiveness, we found that primidone increased RMT, suggesting its action on voltage-gated sodium channels, and the effects on reducing resting and active IO curves, prolonging CSP, enhancing LICI and SAI, and reducing SICI, suggesting interference with of both GABA-B and GABA-A neurotransmitter systems. Propranolol's central effects were restricted on the reducing resting IO curve and enhancing SAI, suggesting noradrenergic modulation of GABA outflow.

Significant repeated measures correlations were observed between tremor amplitude and TMS measures, further implying that proposed mechanisms for both medications are relevant for their anti-tremor effect. Better baseline EBCC predicted a more favorable response to primidone only, underscoring the potential role of relatively preserved cerebellar function in treatment efficacy of primidone. Finally, the presence of ET-plus features was not a significant predictor of treatment response or for TMS measures, implying that ET and ET-plus may share similar pharmacological response and similar neurophysiological signature at the cortical level [41,42]. This finding builds upon a recent study that reported no significant differences in EMG characteristics between ET and ET-plus patients [42].

4.1. Potential mechanisms of action of primidone in ET patients

Primidone effects on corticospinal excitability were evident by increased RMT and decreased rest and active IO curves. Since RMT increases with drugs that block voltage-gated sodium channels but is unaffected by those targeting GABA receptors [15,16], our results support previous assumptions that the non-metabolized form of primidone alleviates tremor by blocking voltage-gated sodium channels [7,15,43,44]. The impact of primidone on AMT did not reach statistical significance, which aligns with previous studies that have reported a more notable increase in RMT compared to AMT with other drugs targeting voltage-gated sodium channels [45]. On the other hand, MEPs elicited by TMS intensities higher than required for motor thresholds (as probed by IO curves), are governed by transsynaptic activation of corticospinal neurons through complex network of excitatory circuits controlled by

Table 2
Estimated marginal means before and after treatment, contrast estimates and standardized effect sizes (Cohen's d) for all outcomes, including non-significant results, with corresponding 95 % CI.

Outcome measure		Treatment	EMMs[a] before	EMMs after	CE[b] (95 % CI)	Cohen's d (95 % CI)
Total TETRAS-P[c] score		Primidone	22.19	19.44	−2.75 (−5.75, 0.24)	−0.59 (−1.21, 0.03)
		Propranolol	22.95	17.27	−5.68 (−2.26, −0.09)	−1.22 (−1.94, −0.49)
TETRAS-P hand tremor[d]		Primidone	18.9	16.45	−2.45 (−4.72, −0.18)	−0.78 (−1.41, −0.16)
		Propranolol	18.92	14.79	−4.13 (−6.76, −1.51)	−1.29 (−2.02, −0.56)
Accelerometry – RMS[e] amplitude		Primidone	0.26	0.2	0.06 (0.01, 0.10)	0.66 (0.20, 1.13)
		Propranolol	0.32	0.22	0.11 (0.04, 0.17)	0.87 (0.33, 1.41)
RMT[f]		Primidone	46.75	51.35	4.60 (1.99, 7.22)	1.31 (0.63, 1.99)
		Propranolol	43.95	44.27	0.32 (−2.09, 2.73)	0.02 (−0.66, 0.78)
AMT[g]		Primidone	39.24	40.82	1.58 (−0.99, 4.14)	0.42 (−0.23, 1.07)
		Propranolol	36.01	37.1	1.09 (−1.50, 3.67)	0.33 (−0.38, 1.04)
MEP max[h] rest input/output curve		Primidone	3.50	1.94	−1.56 (−2.45, −0.67)	−1.56 (−2.35, −0.77)
		Propranolol	3.00	2.69	−0.31 (−1.17, −0.06)	−0.31 (−1.03, 0.40)
AUC[i] rest input/output curve		Primidone	105.18	55.9	−49.28 (−72.08, −26.47)	−1.76 (−2.53, −1.00)
		Propranolol	100.85	75.7	−25.15 (−47.74, −2.55)	−0.79 (−1.52, −0.06)
PS[j] rest input/output curve		Primidone	0.09	0.04	−0.05 (−0.08, −0.02)	−1.42 (−2.18, −0.65)
		Propranolol	0.09	0.07	−0.02 (−0.06, 0.02)	−0.46 (−1.18, 0.26)
MEP max active input/output curve		Primidone	5.83	5.13	−0.71 (−1.85, 0.44)	−0.45 (−1.19, 0.29)
		Propranolol	6.22	6.81	0.59 (−0.65, 1.84)	0.39 (−0.41, 1.18)
AUC active input/output curve		Primidone	391.89	327.09	−64.80 (−150.30, 20.70)	−0.54 (−1.28, 0.19)
		Propranolol	418.11	441.41	23.31 (−68.99, 115.61)	0.19 (−0.59, 0.98)
PS active input/output curve		Primidone	0.14	0.09	−0.05 (−0.09, −0.02)	−1.26 (−2.02, −0.50)
		Propranolol	0.13	0.14	0.00 (−0.04, 0.05)	−0.05 (−0.71, 0.81)
MEP max CSP[k] curve		Primidone	194.47	207.72	13.25 (9.95, 36.45)	0.40 (−0.28, 1.07)
		Propranolol	194.97	192.42	−2.55 (−27.48, 22.39)	−0.15 (−0.85, 0.56)
AUC CSP curve		Primidone	11788.7	13340.6	1551.9 (366.3, 2737.6)	0.92 (0.27, 1.58)
		Propranolol	11962	10844.5	−1117.4 (−2413, 178.2)	−0.69 (−1.38, 0.01)
PS CSP curve		Primidone	2.99	3.25	−0.31 (−0.77, 0.15)	0.27 (−0.38, 0.92)
		Propranolol	2.83	2.52	0.25 (−0.26, 0.77)	−0.47 (−1.17, 0.23)
SICI[l] curve	70 % AMT	Primidone	0.62	0.75	0.13 (−0.08, 0.35)	0.54 (−0.08, 1.17)
		Propranolol	0.74	0.79	0.05 (−0.20, 0.30)	0.20 (−0.47, 0.87)
	80 % AMT	Primidone	0.53	0.72	0.20 (0.06, 0.33)	0.82 (0.20, 1.45)
		Propranolol	0.58	0.62	0.04 (−0.09, 0.17)	0.16 (−0.52, 0.84)
	90 % AMT	Primidone	0.58	0.76	0.18 (0.03, 0.33)	0.71 (0.09, 1.34)
		Propranolol	0.59	0.64	0.05 (−0.09, 0.19)	0.23 (−0.44, 0.90)
	100 % AMT	Primidone	0.56	0.85	0.29 (0.09, 0.49)	1.08 (0.45, 1.71)
		Propranolol	0.6	0.64	0.04 (−0.13, 0.20)	0.15 (−0.52, 0.82)
LICI[m]		Primidone	0.34	0.21	−0.14 (−0.24, −0.03)	−0.76 (−1.41, −0.11)
		Propranolol	0.3	0.35	0.04 (−0.07, 0.16)	0.24 (−0.45, 0.94)
ICF[n]		Primidone	1.39	1.32	−0.07 (−0.34, 0.21)	−0.21 (−0.85, −0.42)
		Propranolol	1.44	1.75	0.32 (−0.07, 0.70)	0.66 (−0.02, 1.34)
SAI[o]	20 ms	Primidone	0.9	0.7	−0.20 (−0.37, −0.04)	−0.69 (−1.32, 0.07)
		Propranolol	0.92	0.69	−0.22 (−0.40, −0.05)	−0.77 (−1.45, −0.01)
	22 ms	Primidone	0.76	0.59	−0.16 (−0.32, −0.01)	−0.67 (−1.31, −0.04)
		Propranolol	0.75	0.74	−0.01 (−0.19, 0.17)	−0.04 (−0.71, 0.63)
	24 ms	Primidone	0.68	0.48	−0.19 (−0.35, −0.04)	−0.95 (−1.58, −0.31)
		Propranolol	0.68	0.61	−0.06 (−0.25, 0.12)	−0.27 (−0.93, −0.40)

[a] EMM: estimated marginal mean.
[b] CE: confidence interval.
[c] TETRAS – P: The Essential Tremor Rating Assessment Scale – Performance.
[d] TETRAS – P hand tremor sub-score: sum of items 4, 6, 7, and 8.
[e] RMS – root mean square.
[f] RMT – resting motor threshold.
[g] AMT – active motor threshold.
[h] MEP max - maximal motor evoked potential.
[i] AUC – area under the curve.
[j] PS – peak slope.
[k] CSP – cortical silent period curve.
[l] SICI - short intracortical inhibition.
[m] LICI – long intracortical inhibition.
[n] ICF – intracortical facilitation.
[o] SAI – short afferent inhibition.

inhibitory circuits. These are regulated by glutamatergic and GABA-Aergic activity, which are in turn modulated by noradrenergic and serotonergic circuits [15]. Given that primidone's metabolite, phenobarbital, acts on GABA-A receptors, this compound is likely responsible for the observed decrease in both the resting and active IO curves.

The overall effects of primidone on intracortical inhibition observed in our study suggest that its impact on tremor is primarily driven by the enhancement of GABA-B intracortical inhibition. Primidone increased duration of CSP and increased LICI, reflecting engagement of GABA- B inhibitory circuits [21]. At the same time, primidone decreased SICI, a TMS measure of GABA-A mediated intracortical inhibition [21]. We argue that the contrasting net effects of primidone on different types of GABAergic intracortical inhibition reflects stronger activation of GABA-B receptors by unmetabolized primidone, compared to activation of GABA-A receptors by its metabolite phenobarbital. SICI and LICI are

mediated by different populations of inhibitory interneurons [46]. Within these intraneuronal inhibitory circuits, GABA-A receptors are primarily located post-synaptically (where they mediate SICI), whereas GABA-B receptors are located both post-synaptically (where they mediate LICI and CSP) and pre-synaptically (where they modulate SICI through presynaptic inhibition) [47]. In the presence of strong GABA-B receptor activation, SICI may be secondarily reduced as a result of diminished GABA release from the interneurons mediating postsynaptic GABA-A inhibition [47]. Therefore, the influence of unmetabolized primidone on GABA-B receptors may have surpassed the impact of phenobarbital on GABA-A receptors. Similar opposing effect on CSP, LICI and SICI were reported for two other GABA-Bergic medications pregabalin and tiagabine [47,48]. Finally, primidone increased SAI, a measure of sensory-motor inhibition that is mediated by the central cholinergic pathway [15]. Since there are no previous indications that primidone directly affect cholinergic receptors, we propose an indirect mechanism [49,50,51]. SAI is modulated by at least two different GABA-A circuits: one that increases SAI, and another that decreases acetylcholine release, consequently reducing SAI [49]. Thus, primidone' effect on SAI can be attributed to its action of its metabolite phenobarbitone on the GABA-A receptor subtype in the inhibitory SAI circuit [49].

4.2. Potential central mechanisms of action of propranolol in ET patients

While it is widely accepted that the effectiveness of propranolol in ET is due to its blockade of peripheral beta-2 receptors in muscle spindles [52], the complete mechanism underlying its anti-tremor effects remains unclear, with potential central effects also being suggested [6,7]. The human motor cortex is innervated by ascending noradrenergic projections from locus coeruleus [50], where increased synaptic concentrations of noradrenaline may shape motor behavior by its neuro-modulatory effects on cortical excitability [51]. We therefore employed TMS to examine the effects of propranolol on corticospinal excitability and various measures of intracortical inhibition. Unlike primidone's pronounced effect on corticospinal excitability, propranolol reduced the resting IO curve only, and to a lesser extent than primidone (the confidence interval for the effect of propranolol on the AUC of the resting IO curve was broad, with the lower boundary indicating smaller effect size compared to primidone). The effect on rest IO curve is likely mediated indirectly through noradrenergic modulation of GABA outflow [53] and is consistent with a recent study that also observed a decrease in the IO curve among ET patients treated with propranolol [54]. Furthermore, we found no evidence that propranolol influences any measures of GABA-A or GABA-B intracortical inhibition, as probed by SICI, CSP and LICI. This aligns with previous pharmacological TMS studies, which suggest that noradrenergic modulation of these intracortical measures primarily depends on α2 receptors, and to a lesser extent, α1 receptors—both of which are not significantly affected by propranolol [50–52] Nevertheless, propranolol increased SAI, a cholinergic depended measure of afferent inhibition, that is modulated by noradrenergic circuits via GABAergic circuits [49,55,56].

The reason propranolol preferentially increased SAI at an ISI of 20 ms is uncertain. However, consistent with our results, a recent study in healthy controls demonstrated a decrease of SAI at an ISI of 20 ms with a noradrenaline reuptake inhibitor [56], suggesting that afferent inhibition at shorter ISIs may be specifically sensitive to noradrenergic modulation.

4.3. Reconciling the mechanisms of central tremor-modulating properties for primidone and propranolol

The significant correlations between alterations in TMS parameters and shifts in tremor severity suggest that the detected neurophysiological changes are intricately linked to the tremor improvement. For primidone, the decrease in corticospinal excitability (as evidenced by the decrease in RMT and reduction of parameters of the rest and active IO curves), the increase in CSP duration, and the enhancement of SAI, correlated with a decrease in tremor severity. This supports the argument that primidone exerts its anti-tremor effect by blocking voltage-gated sodium channels and through the engagement of GABA-A and GABA-B inhibitory circuits, consistent with the GABA hypothesis of ET [9,57,58]. Recently, extra synaptic GABA-A receptors that regulate tonic inhibition were ascribed an important role in the pathophysiology of ET [55]. Given that barbiturates are known activators of these receptors [16], it is possible that the anti-tremor effect of primidone, through its metabolite phenobarbital, also occurs via extra-synaptic GABA-A receptors. However, reliable TMS measures of extra-synaptic GABA-A activity are currently not available [56].

Although the effects of propranolol on TMS measures were less pronounced than those of primidone, its influence on corticospinal excitability and SAI, coupled with the correlations of these measures with tremor improvement, indicate that the central action of propranolol, via noradrenergic modulation of GABAergic circuits, contributes to its effectiveness in essential tremor.

4.4. EBCC as a predictor of treatment effectiveness in ET

Both primidone and propranolol are effective only in a proportion of patients with ET, with currently no known predictors of response [7,59]. We therefore investigated if degree of impairment in EBCC may predict the therapeutic response. Animal studies have shown that cerebellar cortex, cerebellar nuclei and inferior olives are the critical neuroanatomical sites for acquisition, timing and retention of conditioned responses in EBCC paradigm [19,60]. Patients with ET have conditioning deficits, supporting the hypothesis that ET is caused by a disturbance of olivo-cerebellar circuits [19,20]. We found that better baseline EBCC predicted better response to primidone, suggesting that sufficient preservation of cerebellar function might be needed for primidone to exert its action. A reduction in GABA-A and GABA-B receptors in the dentate nucleus of the cerebellum has been documented post-mortem in individuals with ET [61]. Our results suggest that primidone's effectiveness in ET may be partly related to its binding to GABA-ergic receptors in the cerebellum. The absence of a predictive effect of EBCC on the response to propranolol may be attributed to its primary peripheral anti-tremor activity, which operates effectively and independently of alteration in central circuits that involves cerebellum.

4.5. Study limitations

Our study has several limitations that stem from its observational design. The selection of medication and determination of dosages were not under the control of the investigator but were instead based on the clinical decisions of the treating neurologist and patient preferences. As the result, the doses received were on the lower end compared to the doses used in interventional clinical studies, reflecting the real-world scenarios, where patients may be hesitant to increase the dose to avoid potential side effects [7]. Nonetheless, the response rate and the extent of tremor reduction in our study are consistent with reports from previous studies, indicating that the attained clinical effect was sufficient for studying drug mechanisms. However, the narrow range of dosages in our cohort limited our ability to assess potential dose-related effects of primidone and propranolol on TMS measures. Patient groups receiving primidone and propranolol were not matched based on age, disease duration, gender, the presence of "plus" features, side of stimulation (dominant or non-dominant hemisphere), which introduced potential confounding variables. To address this issue, these parameters were incorporated as covariates in the GLMM, aiming to mitigate the influence of demographic and clinical variations among the subjects and thereby ensure that any detected effects were not merely attributable to these differences. Subtle cognitive decline [62] and questionable bradykinesia [63] are often associated with ET. Although recent studies

suggests that ET and ET-plus patients are electrophysiologically similar [42], the lack of systematic evaluation of bradykinesia in our study limited our ability to assess this "plus" feature as a potential confounder. The absence of cognitive assessment may be particularly relevant for interpretation of changes in SAI, since this measure dependents on central cholinergic function. Neither the patients or the investigators were blinded to the treatment. To mitigate this bias on clinical tremor ratings and subjective VAS scores, we included only accelerometry data in the correlation analysis. The lack of a placebo control group limits our ability to distinguish the specific effects of the medications from potential placebo effects. Nevertheless, previous studies confirmed that the clinical effectiveness of both propranolol and primidone is superior to placebo [7]. Regarding the changes in TMS measures observed in our study, we believe they are unlikely to be placebo-induced, given the clear electrophysiological differences found between primidone and propranolol, despite similar clinical responses. We experienced substantial dropout during the study, which may have impacted our ability to detect significant effects. However, we utilized GLMM statistics to analyze data from all patients, including the ones who only completed the first visit, thus adhering to the intention-to-treat principle. Nevertheless, according to our post-hoc power analysis, larger sample size might have allowed us to identify the medium effects of primidone on MEP max and AUC of the active IO curve, as well as the medium effect of propranolol on ICF, as statistically significant. Additionally, the confidence interval for the effect of propranolol on the AUC of the resting IO curve was broad, with the lower boundary indicating a very small effect size. Therefore, a larger sample size may be needed to more accurately assess the significance of propranolol's effect on the resting IO curve. Cerebellar function was assessed using EBCC only, but additional insights could have been gained by investigating cerebellar-cortical activity through TMS measures. The utilization of TMS for exploring the central nervous system effects of medications is also subject to some limitations, including susceptibility of TMS to external and internal influences that may introduce variability into the results [64]. However, the within-subject design comparing TMS measures before and after medication in the same patients provides valuable insights into drug-induced changes. Another limitation is the absence of a neuronavigational system for TMS targeting, which could introduce variability in cortical stimulation location. To maintain consistency, the motor "hotspot" was marked with a skin marker on the participant's head and kept constant throughout the experiment, with the procedure repeated in the following session. Finally, a set of TMS measures examined in our study provides insights into drug-induced changes at the system level of the motor cortex. These changes represent the net effect of various modifications at both cortical and subcortical levels, and therefore, do not provide information on the specific circuit targeted by the drug.

5. Conclusions

Primidone operates in vivo to mitigate ET by inhibiting voltage-gated sodium channels and engaging with GABA-A intracortical circuits, aligning with prior preclinical hypotheses. The results on CSP, LICI and SICI indicate even stronger involvement of intracortical GABA-B intracortical circuits with primidone, marking a novel discovery in our study. Primidone was more effective in those patients with more preserved EBCC, suggesting its efficacy could also be attributed to binding to GABAergic receptors in the cerebellum. The influence of propranolol on the rest IO curve and SAI implies that, beyond its well-known peripheral effects, central action also plays a role in its effectiveness in ET. While caution is warranted when drawing conclusions about underlying mechanisms, this in vivo study serves as a valuable complement to preclinical research, enhancing our understanding of the mechanisms on how anti-tremor medications work.

Data availability statement

Anonymized data used for this study are available upon reasonable request from the corresponding author.

Declarations

Standard protocol approvals, registrations, and patient consents.

The study was approved by the Republic of Slovenia National Medical Ethics Committee. Written informed consent was obtained from all participants according to the Declaration of Helsinki. The study was registered with ClinicalTrials.org (NCT04692844).

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CRediT authorship contribution statement

Katarina Vogelnik Žakelj: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Neža Prezelj:** Writing – review & editing, Investigation. **Milica Gregorič Kramberger:** Writing – review & editing, Data curation. **Maja Kojović:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.parkreldis.2024.107151.

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