RESEARCH ARTICLE

Frequency-Selective Suppression of Essential Tremor via Transcutaneous Spinal Cord Stimulation

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ABSTRACT: Background: Essential tremor (ET) is a common debilitating condition, yet current treatments often fail to provide satisfactory relief. Transcutaneous spinal cord electrical stimulation (tSCS) has emerged as a potential noninvasive neuromodulation technique capable of disrupting the oscillatory activity underlying tremors.
Objective: This study aimed to investigate the potential of tSCS to disrupt tremor in a frequency-dependent manner in a cohort of patients with ET.
Methods: Eighteen patients with ET completed the study. The experiment consisted of 60-s postural tremor recording, during tSCS at tremor frequency, at 1 Hz, at 21 Hz, no stimulation, and trapezius stimulation. Tremor frequency and amplitude were analyzed and compared across the conditions.
Results: We found tremor amplitude reduction at tremor frequency stimulation significant only during the second half of the stimulation. The same stimulation resulted in the highest number of responders. tSCS at 1 Hz showed a trend toward decreased tremor amplitude in the latter half of stimulation. tSCS at 21 Hz did not produce any significant alterations in tremor, whereas trapezius stimulation exacerbated it. Notably, during tremor frequency stimulation, a subgroup of responders exhibited consistent synchronization between tremor phase and delivered stimulation, indicating tremor entrainment.
Conclusions: Cervical tSCS holds promise for alleviating postural tremor in patients with ET when delivered at the subject's tremor frequency. The observed changes in tremor amplitude likely result from the modulation of spinal cord circuits by tSCS, which disrupts the oscillatory drive to muscles by affecting afferent pathways or spinal reflexes. However, the possibility of an interplay between spinal and supraspinal centers cannot be discounted. © 2024 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: essential tremor; tSCS; entrainment; spinal cord; noninvasive stimulation

Essential tremor (ET) presents a significant burden for patients, not only because of the functional limitations caused by tremor but also because of the associated social stigma. Its exact prevalence remains challenging to estimate precisely, yet it has often been regarded as the most prevalent movement disorder.[1] Despite this, many aspects of ET remain poorly understood, as illustrated by its recent reclassification as a syndrome, phenomenologically and pathophysiologically heterogeneous and caused by multiple etiologies, rather than a single entity.[2,3]

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The lack of a comprehensive understanding of ET has a negative reflection on our capability to treat it. Current first-line treatments primarily consist of symptomatic oral medications,[4] which provide tremor relief only to a minority of patients (30%–70%)[5,6] and often to a limited extent.[7] Furthermore, about half of the patients eventually discontinue oral medications because of side effects,[8,9] particularly among the elderly, who are most commonly affected by ET.[10] Other treatment options include brain surgery (such as deep brain stimulation [DBS] and magnetic resonance imaging–guided focused ultrasound) or botulinum toxin injections; nevertheless, they are not always feasible or they are perceived as overly invasive by patients. For all these reasons, the treatment of ET is still an unmet need.

Although current pharmacological treatments are not specifically tailored for ET,[4] the surgical procedures have been developed with the intent to target the putative mechanisms causing this condition.[11] The exact pathophysiology of ET is not fully elucidated, but most research studies recognize the cerebello-thalamo-cortical (CTC) loop as the pivotal substrate for various forms of tremor, including ET.[12] It is generally acknowledged that the pathophysiologic hallmark of action tremor is oscillations in the CTC loop, which are coupled with muscle activity that results in visible tremor.[13] In ET these pathologic oscillations seem to be driven by the cerebellum, as supported by several pieces of evidence,[14-16] including the idea that ventralis intermediate nucleus DBS works by disrupting pathologic information flow propagating oscillatory signals through the network and producing tremor.[17]

A noninvasive way to interfere with brain oscillatory activity is by applying noninvasive electrical stimulation to the central or peripheral nervous system. For instance, a previous study demonstrated that alternating current stimulation, applied over the cerebellum and phase-locked to the tremor, can alter cerebellar activity, causing a reduction of the tremor amplitude in ET.[18] Peripheral nerve stimulation, applied with different protocols, has also shown promising results.[19] In this case, because tremor is sustained partly by afferent feedback,[20] the rationale is to interfere with the afferent inputs with the intent to reduce tremor.[19] All this evidence seems to suggest that the perturbation of the flow of the oscillatory activity driving tremor, at different levels of its propagation (from its source to the muscles), can potentially suppress it.

Transcutaneous spinal cord electrical stimulation (tSCS) is an emerging modality for noninvasive neuromodulation of the central nervous system that has minimal adverse effects. To date, tSCS has been applied mostly as a low-intensity direct current over the spinal cord,[21] mainly with the intent to reduce spasticity and improve α-motor neuron recruitment[22] in patients with spinal cord injury,[23] stroke,[24] and hereditary spastic paraplegias.[25] However, similarly to transcranial electrical stimulation, tSCS can be delivered as bursts of biphasic current pulses at specific frequencies, which could potentially interfere with the pathologic tremorgenic drive to the spinal motoneurons. Moreover, tSCS is thought to target dorsal roots,[26,27] and the principle of tremor suppression by peripheral nerve stimulation might apply also to this technique.

In this study, we explored for the first time the potential of tSCS to modify the propagation of tremor to the muscles in ET syndrome. tSCS was delivered in an open-loop fashion (ie, stimulation was not adapted to the tremor characteristics), but at different frequencies, to assess whether specific stimulus frequencies determine tremor changes. In addition, using tremor frequency as one of the stimulation conditions, we could test whether possible phase alignment between stimulation and tremor (ie, tremor entrainment) could predict tremor amplitude changes or show other interactions between stimulation and endogenous neural oscillations causing tremor.

In summary, we aimed to investigate whether tSCS can perturbate postural tremor in ET, induce oscillatory neural entrainment, and suppress tremor, thereby exploring the feasibility of tSCS as a therapeutic intervention for ET.

Subjects and Methods

Participants

Nineteen participants (mean age, 73.9 ± 8.7 years; six females) diagnosed with idiopathic ET syndrome (either ET or ET plus)[2] were recruited from the movement disorders outpatient clinic of the National Hospital for Neurology and Neurosurgery (Queen Square, London, UK). Participants were excluded if they had very mild and/or intermittent tremor that could not be properly recorded, if they had a cardiac pacemaker or other electronic implants, or if they were unable to maintain the arm outstretched for the required time. All subjects participated in a single visit of approximately 2-hour duration. Participants were asked to refrain from taking their oral treatment for tremor the evening before the experiment; for patients receiving therapy with botulinum toxin, the recording occurred at least 3 months after the last injection. The experimental protocol was approved by the local institutional review board (REC Number 03/N018) and conducted in accordance with the Declaration of Helsinki. All participants gave written informed consent before starting the experiment.

Clinical Assessment

Demographic and clinical data were collected (Table 1). Tremor severity was assessed by a neurologist expert in movement disorders using the Fahn-Tolosa-Marin Tremor Rating Scale (FTM). The most affected hand, in

TABLE 1 *Clinical features and stimulation parameters*

Subject	Sex	Age (y)	HT	Diagnosis	DD (y)	Treatment	FTM	MT (mA)	SI (mA)	TF (Hz)
1	M	70	R	ET+ (Re)	25	None	67	110	55	5
2	M	43	L	ET+ (Re, D)	20	CLZ	20	53	33	6
3	M	72	R	ET	10	None	14	80	70	4.8
4	F	77	R	ET+ (Re, D)	8	None	44	70	55	6
5	M	79	L	ET+ (Re, D)	15	BTX	40	90	40	6. 1
6	F	70	R	ET	20	Propranolol	18	100	30	4.8
7	M	71	R	ET	5	Propranolol	22	100	50	5.7
8	M	86	R	ET	27	Bisoprolol	39	90	70	5
9	F	74	R	ET	35	BTX	44	60	50	4.8
10	M	78	R	ET+ (Re)	12	None	23	70	60	6
11	F	77	R	ET+ (Re, D)	40	BTX, propranolol	59	140	40	4.8
12	F	81	L	ET+ (Re)	10	None	30	90	35	5.5
13	F	73	R	ET+ (D, A)	14	BTX	23	100	60	5.5
14	M	73	L	ET	20	None	24	100	80	5.3
15	M	79	R	ET+ (Re, D)	10	Propranolol	28	–	40	5.8
16	M	82	R	ET+ (Re, D)	40	BTX	65	60	40	4.2
17	M	75	L	ET	5	BTX, propranolol	15	70	30	4.6
18	M	76	L	ET+ (Re, D)	15	Gabapentin, BTX	16	70	45	5
Average ± SD		74.2 ± 8.8			18.4 ± 11.1		32.8 ± 17.1	85.5 ± 22.1	49.1 ± 14.7	5.3 ± 0.6

Abbreviations: HT, hand tested; DD, disease duration; FTM, Fahn-Tolosa-Marin Tremor Rating Scale; MT, motor threshold; SI, stimulation intensity; TF, tremor frequency; M, male; R, right; ET+, essential tremor plus; L, left; Re, resting tremor; D, questionable dystonia; CLZ, clonazepam; ET, essential tremor; F, female; BTX, botulinum toxin injections; A, questionable ataxia.

case of asymmetric tremor, or the dominant hand, in case of symmetric tremor, was selected for stimulation.

Experimental Design

The experimental design is detailed in the Supporting Information. In brief, the protocol consisted of five conditions: (1) no stimulation (baseline), tSCS delivered at (2) tremor frequency (Tremor-Stim), at (3) 1 Hz, at (4) 21 Hz, and (5) stimulation over the trapezius muscle (Trap-Stim), which served as control. During the stimulation, the electrode serving as cathode was placed between C5 and C6 spinous processes or over the upper trapezius muscle (Trap-Stim), while two stimulation electrodes serving as anode were placed over each clavicle. To avoid recruitment of motoneurons (presumably via transsynaptic pathways from the dorsal to the ventral horn), which would interfere with motor control of the upper limbs, stimulation intensity was set below the motor threshold (−10 mA). Tremor was recorded at baseline and during each stimulation by a triaxial accelerometer placed on the dorsum of the hand most affected by tremor. The experiment consisted of 60 s tremor recording (block), during which patients had to hold their arms outstretched and pronated, at each stimulation condition (Fig. 1); this was repeated four times, and the order of the blocks was randomized.

Data Analysis and Statistics

Details are given in the Supporting Information. Acceleration in the z-axis was used to estimate the tremor amplitude.[28,29] To normalize the recorded epoch tremor amplitudes, they were z scored using the mean and standard deviation of the tremor amplitudes obtained in the baseline blocks. The tremor frequency was estimated for each 1-s epoch within a block. Similarly to amplitudes, z score normalization was used for the estimated frequencies. Statistics for tremor amplitude and frequency were computed in three periods: the entire block (60 s), the first half of the block (1–30 s), and the second half of the block (31–60 s). In addition to the z scores, results were reported as a relative reduction of tremor as a percent of baseline (the baseline tremor amplitude or frequency was subtracted from those of the different tSCS conditions and divided by average baseline tremor). Linear-mixed model analysis was used to explore the differences in tremor amplitude and frequency (z scores) at the group level

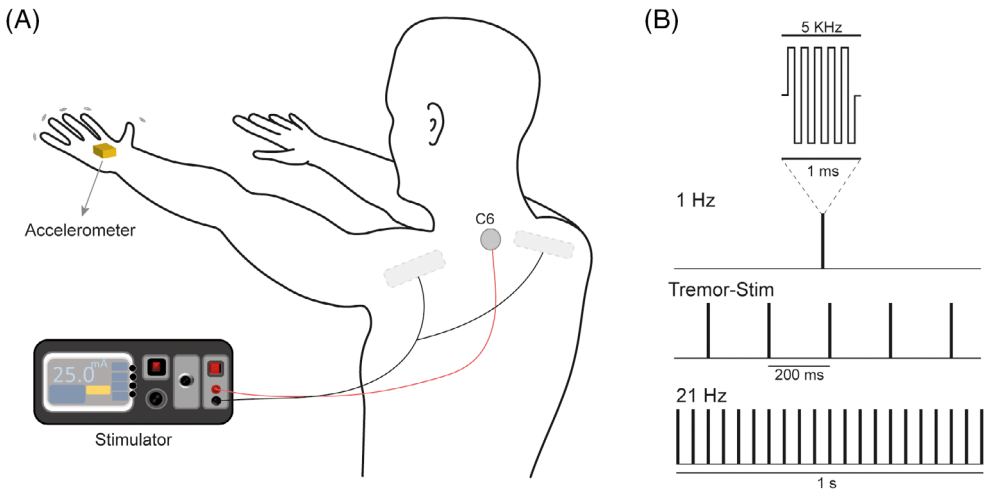

FIG. 1. Experimental setup. (**A**) Electrode location and posture held by the subject during stimulation. (**B**) Transcutaneous spinal cord electrical stimulation (tSCS) waveforms used. As an example, a frequency of 5 Hz is used in this case for the Tremor-Stim condition. [Color figure can be viewed at wileyonlinelibrary.com]

elicited by the different stimulation protocols, using the stimulation condition as fixed effects, and the subject as random effect. *P* values were computed using post hoc tests with Bonferroni correction for multiple comparisons (ie, differences were considered significant for equivalent $P < 0.005$). To investigate the subject-specific responsiveness to the stimulation protocols, we used Wilcoxon ranked tests with Bonferroni correction to analyze tremor amplitude and frequency across the 1-s segments across stimulation conditions because normality assumption of the data was not satisfied.[18]

We also analyzed the synchronization between the tremor phase and the phase of the stimulation in the Tremor-Stim blocks. The phase difference between the acceleration and the modulating signal used to generate the stimulation was computed for this purpose. The phase locking value (PLV) was used to estimate the synchronization between the two signals. The statistical threshold to determine significant PLV levels for each subject was defined as the mean value of the PLV levels obtained from the baseline blocks plus three standard deviations across subjects. Circular correlation was used to study the relationship between the mean phase difference and the PLV. Finally, to characterize the relationship between the effects of stimulation and tremor features, we calculated the Pearson's correlation coefficient between the following variables across conditions: *z* scores estimating change in tremor amplitude and frequency, absolute values of tremor amplitude and frequency at baseline, PLV during Tremor-Stim, age, FTM scores, motor threshold, and stimulation intensity.

Results

Results are briefly summarized in this section, whereas details can be found in the Supporting Information. Eighteen patients completed the study; one subject was excluded from the analysis because the experimental task was not completed because of arm fatigue induced by prolonged and repeated maintenance of the outstretched posture. Clinical features and stimulation parameters are summarized in Table 1.

Fig. 2A shows the evolution of the tremor amplitude (average across subjects) during the different stimulation blocks. When considering the entire stimulation period of 60 s, Tremor-Stim condition resulted in a reduction of tremor amplitude (z score $= -0.33 \pm 0.90$; $-13\% \pm 42\%$ tremor reduction) (Fig. 2B,E). To account for a potential buildup effect in tremor modulation (in line with previous similar studies[18] and compatible with the trends displayed in Fig. 2A), we also analyzed separately the changes in tremor amplitude during the first and second 30-s periods of each block. During the first 30 s (Fig. 2C,F), Trap-Stim resulted in a significantly higher tremor amplitude (z score $= 0.741 \pm 1.88$; $50\% \pm 134\%$ tremor aggravation) than 1-Hz stimulation (z score $= -0.153 \pm 0.90$; $P = 0.022$; $2\% \pm 43\%$ tremor aggravation). In the second 30-s period of the stimulation, Tremor-Stim resulted in a significant tremor reduction (z score $= -0.63 \pm 0.90$; $-25\% \pm 35\%$ tremor reduction) compared with baseline (z score $= 0.05 \pm 0.31$; $P = 0.005$), Trap-Stim (z score $= 0.35 \pm 1.34$; $P < 0.001$; $18\% \pm 65\%$ tremor aggravation), and 21-Hz (z score $= -0.05 \pm 0.80$; $P = 0.024$; $-4\% \pm 29\%$ tremor reduction) conditions (Fig. 2A,D,G).

The Tremor-Stim condition elicited a statistically significant increase in the tremor frequency in the 60-s window (z score $= 0.29 \pm 0.88$; $4\% \pm 12\%$ tremor frequency increase) compared with baseline ($P = 0.013$) and 21-Hz conditions (z score $= -0.02 \pm 0.29$; $P = 0.005$; $0\% \pm 3\%$ tremor frequency change) (Fig. 3A,B,E). A significant increase in tremor frequency was also found during

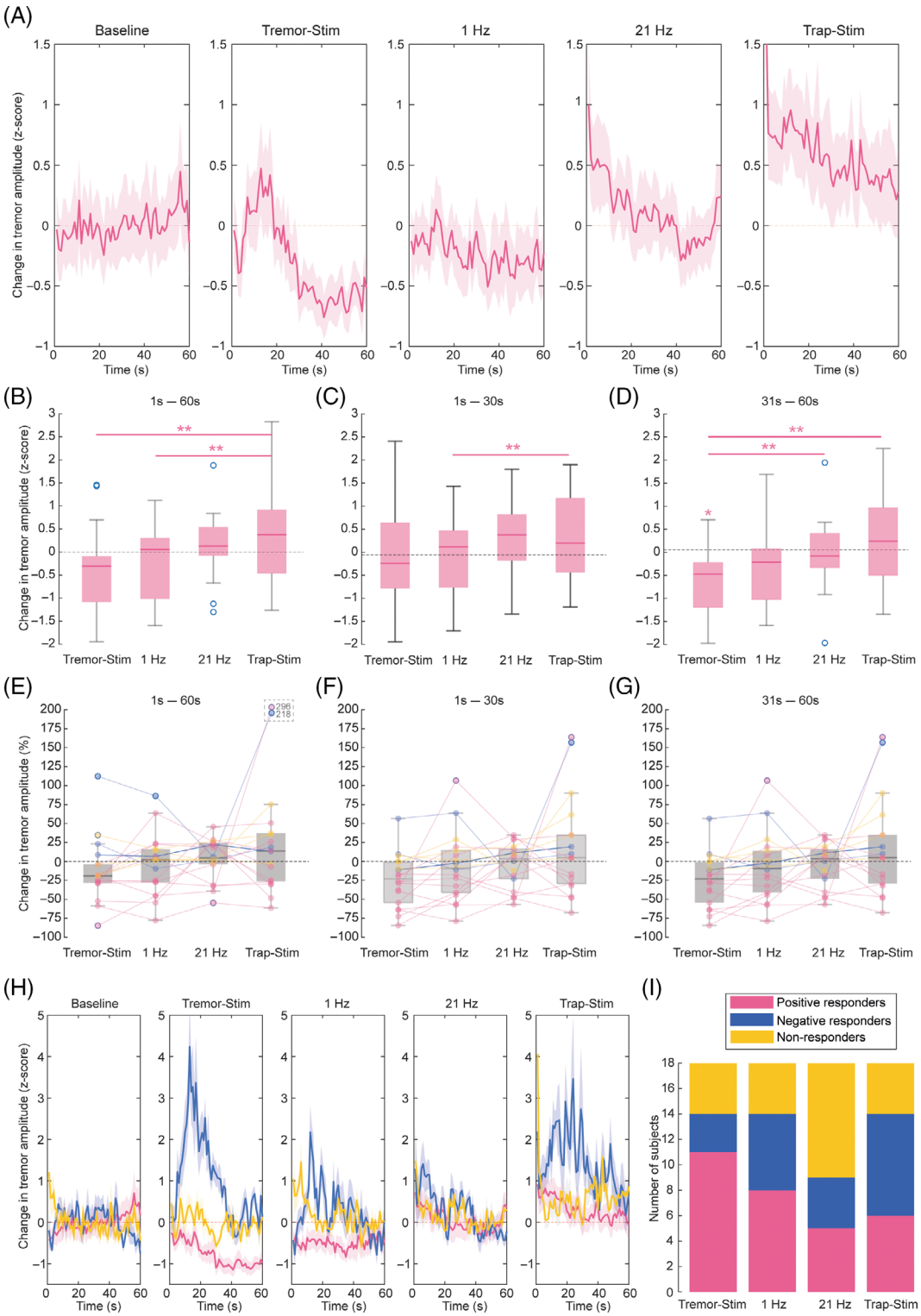

FIG. 2. Transcutaneous spinal cord electrical stimulation (tSCS) effects on tremor amplitude. (**A**) Tremor amplitude traces during the 60-s stimulation period averaged across subjects (mean ± standard error). (**B**–**D**) Change in tremor amplitude normalized to baseline (*z* scores) during the 60-s stimulation block (B); the first half of the stimulation block from second 1 to 30 (C); and the second half of the stimulation block from second 31 to 60 (D). (**E**–**G**) Change in tremor amplitude relative to baseline (%) during the 60-s stimulation block (E); the first half of the stimulation block from second 1 to 30 (F); and the second half of the stimulation block from second 31 to 60 (G). Boxes represent the interquartile range, horizontal line the median value, whiskers 1.5 times the interquartile range, horizontal dashed line the baseline condition, and dots individual participants (pink represents positive responders to Tremor-Stim, yellow represents nonresponders, and blue represents negative responders). (**H**) Tremor amplitude traces (mean ± standard error) during the 60-s stimulation period averaged across subjects and grouped by positive responders (pink line), nonresponders (yellow line), and negative responders (blue line). (**I**) Number of subjects per condition identified as positive responders (pink bar), nonresponders (yellow bar), and negative responders (blue bar). *P < 0.05 between baseline and the target condition, **P < 0.05 between the conditions. [Color figure can be viewed at wileyonlinelibrary.com]

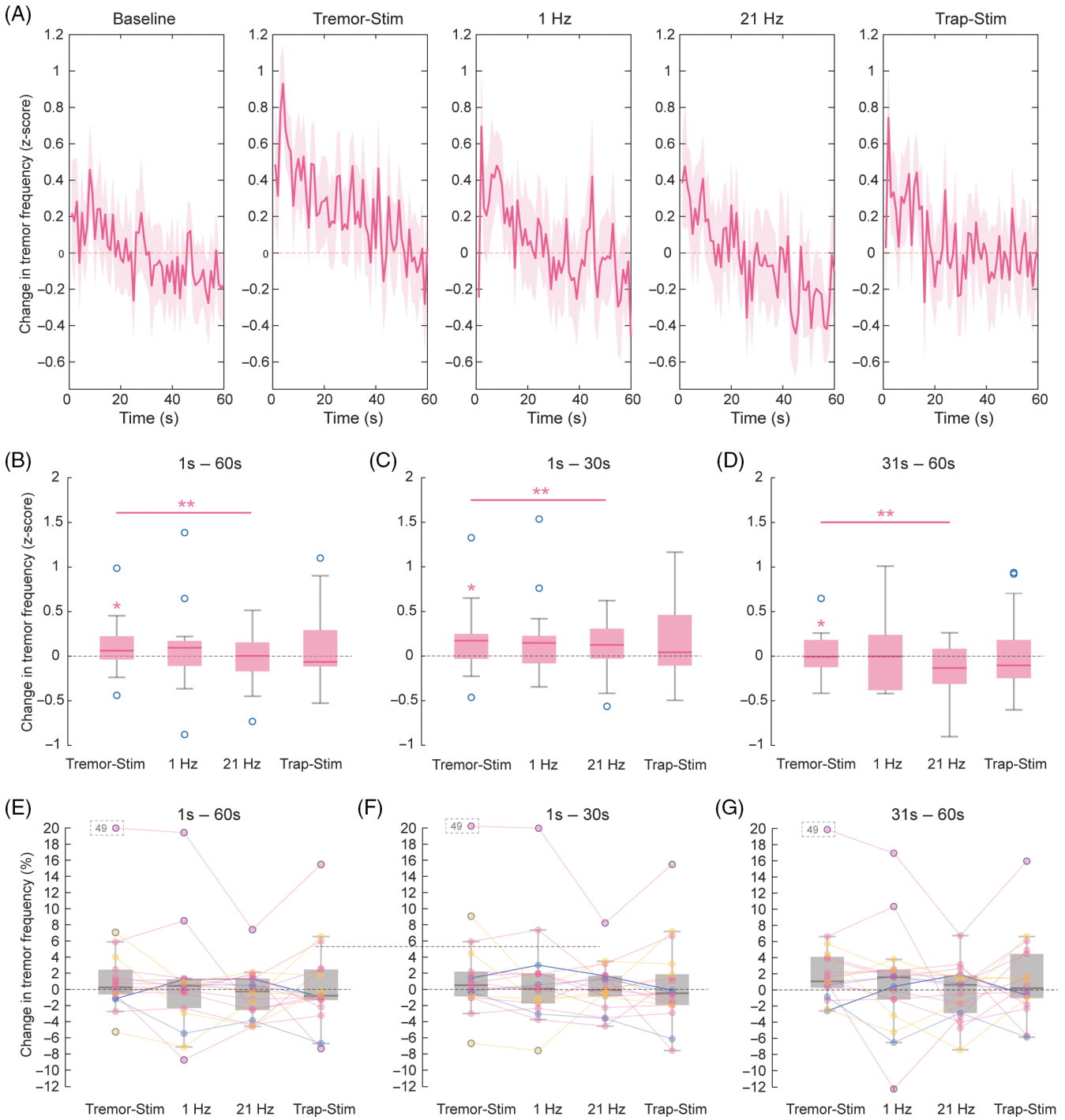

FIG. 3. Transcutaneous spinal cord electrical stimulation (tSCS) effects on tremor frequency. (**A**) Tremor frequency traces during the 60-s stimulation period averaged across subjects (mean ± standard error). (**B–D**) Change in tremor frequency normalized to baseline (*z* scores) during the 60-s stimulation block (B); the first half of the stimulation block from second 1 to 30 (C); and the second half of the stimulation block from second 31 to 60 (D). (**E–G**) Change in tremor frequency relative to baseline (%) during the 60-s stimulation block (E); the first half of the stimulation block from second 1 to 30 (F); and the second half of the stimulation block from second 31 to 60 (G). Boxes represent the interquartile range, horizontal line the median value, whiskers 1.5 times the interquartile range, horizontal dashed line the baseline condition, and dots individual participants (pink represents positive responders to Tremor-Stim, yellow represents nonresponders, and blue represents negative responders). * Indicates *p*-value < 0.05 between Baseline and the target condition; ** indicates *p*-value < 0.05 between the conditions. [Color figure can be viewed at wileyonlinelibrary.com]

Tremor-Stim for the first 30-s period of stimulation (z score = 0.38 ± 0.92; 3% ± 12% tremor frequency increase) compared with baseline ($P = 0.046$) and 21-Hz stimulation conditions (z score = 0.11 ± 0.32; $P = 0.041$; 0% ± 3% tremor frequency change) (Fig. 3C,F). Similar results were obtained during the last 30-s period (Fig. 3D,G).

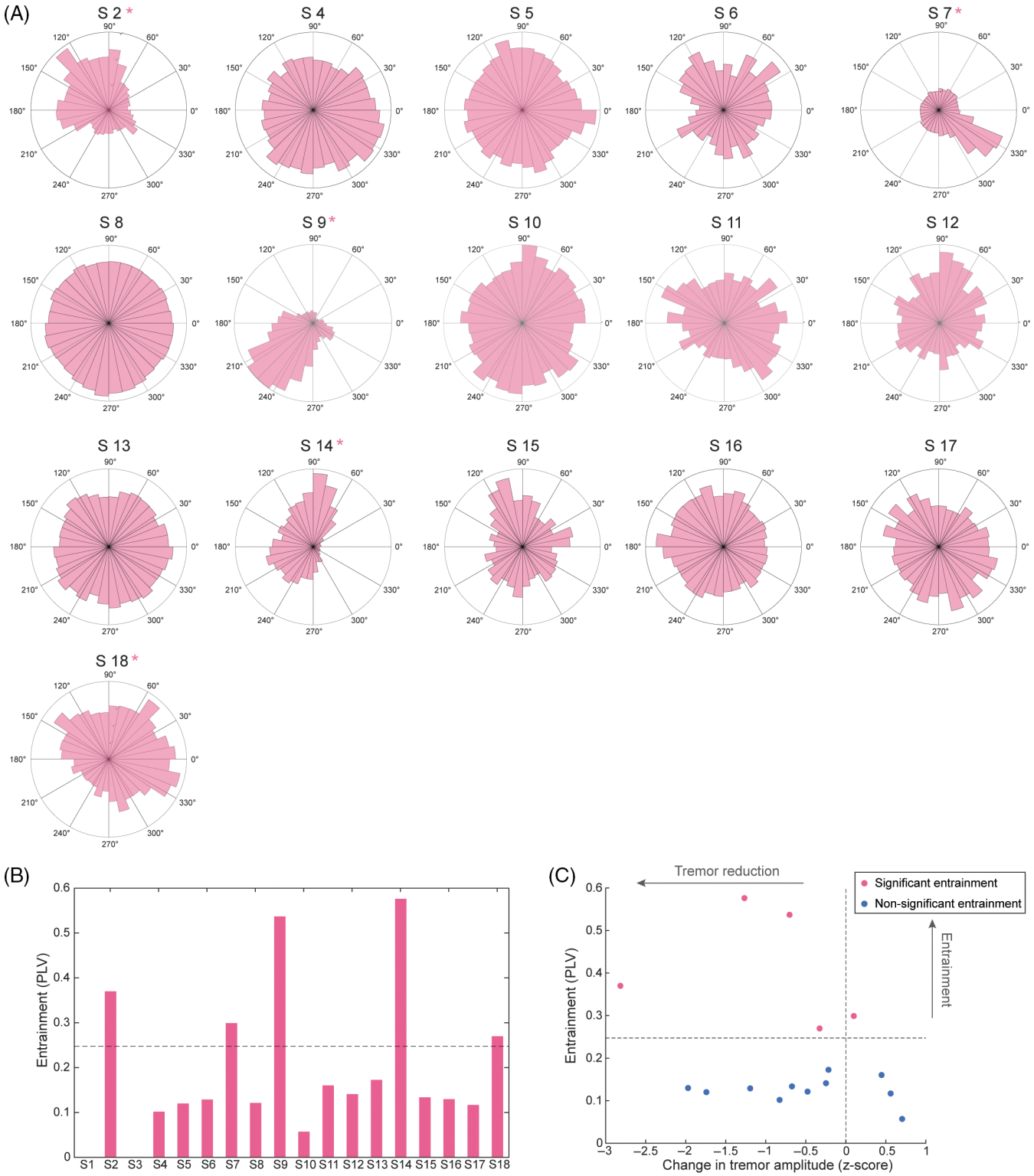

FIG. 4. Tremor entrainment by transcutaneous spinal cord electrical stimulation (tSCS). (**A**) Polar histograms with the phase difference between the tremor and stimulation waveform for the Tremor-Stim (frequency-locked condition). (**B**) Phase locking value (PLV) for each patient. Dotted horizontal line represents the threshold for statistical significance (subjects marked with an asterisk [*] in A). (**C**) Change in tremor amplitude normalized to baseline versus PLV (level of entrainment). The horizontal dashed line represents the statistical threshold for PLV significance, whereas the vertical dashed line corresponds to absence of change in tremor amplitude. Pink dots indicate the subjects with statistically significant phase synchronization by the stimulation, whereas blue dots represent subjects where tremor phase was not significantly modulated. [Color figure can be viewed at wileyonlinelibrary.com]

Patient-specific responsiveness to the different stimulation conditions was also analyzed.[18] z scores of tremor amplitude computed from the second half of the stimulation blocks were compared against the baseline condition. According to their response to the stimulation, patients were assigned to one of three possible groups: positive responders (tremor amplitude decreased statistically significantly during stimulation), negative responders (tremor amplitude increased statistically significantly), and neutral responders (tremor amplitude was left unchanged). Tremor-Stim resulted in the highest number of positive responders (11 subjects), with 3 subjects identified as negative responders and 4 subjects as neutral responders (Fig. 2H,I).

The phase difference between the accelerometric signal and the stimulus was computed across the Tremor-Stim blocks for all subjects (Fig. 4A). Five of 16 subjects showed statistically significant PLVs, meaning the tremor phase was consistently synchronized with the delivered stimulation (Fig. 4B). We did not find any relationship between the mean phase difference and the strength of the synchronization (PLV) at the group level (circular correlation = 0.21; $P = 0.69$), nor at the subgroup level considering only subjects showing significant synchronization (circular correlation = 0.87; $P = 0.14$). Interestingly, higher levels of PLV were associated with tremor reduction: in four of the five subjects showing a significant phase locking with the stimulation, the average tremor amplitude measured during the Tremor-Stim blocks was smaller than the tremor in the baseline blocks (Fig. 4C).

Finally, to explore potential relationships between the effects of tSCS and tremor features, we performed linear correlation analysis between pairs of variables across the different stimulation conditions (Fig. 5). A positive linear relationship (rho = 0.72) between the tremor amplitude and the FTM scores was present. No influence of chronic botulinum toxin treatment on tSCS responsiveness was observed in this study because three of seven patients receiving botulinum toxin were responsive to the Tremor-Stim tSCS protocol.

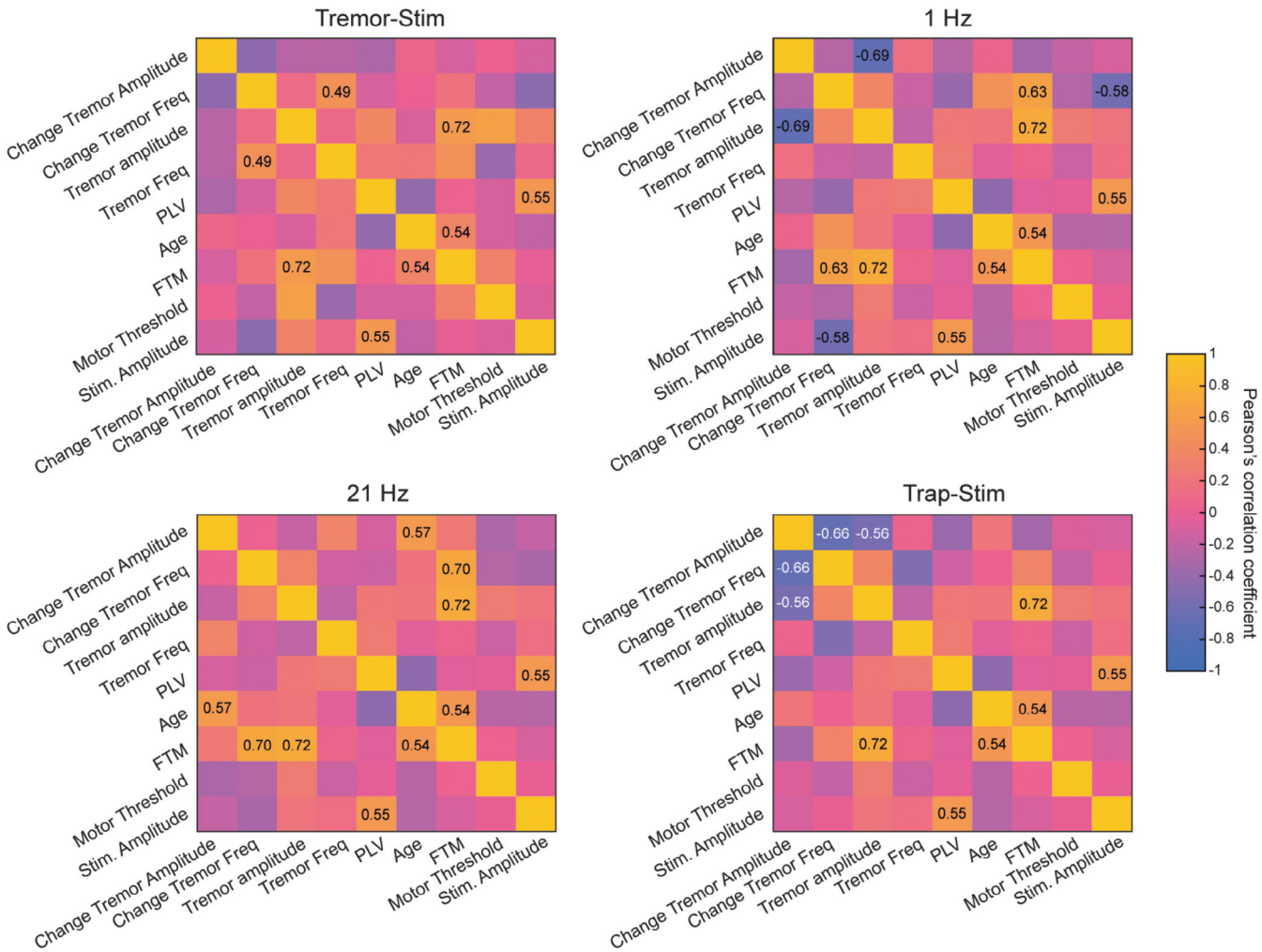

FIG. 5. Correlation matrix between pairs of variables. Linear correlation coefficients are displayed only for those greater than the statistical threshold for significance ($P > 0.05$). [Color figure can be viewed at wileyonlinelibrary.com]

Moreover, there was no difference in the response to tSCS between ET and ET plus (see Supporting Information).

Discussion

In this study, for the first time, we explored the impact of cervical tSCS in disrupting postural tremor in ET syndrome. Our findings showed a significant reduction in tremor amplitude when stimulation was administered at a similar frequency of tremor itself. This result was confirmed by the observation that the same stimulation condition resulted in the highest number of responders (11/18) compared with the other conditions. Of particular interest, we noted that among responders, there was a consistent synchronization between tremor phase and the delivered stimulation, suggesting an entrainment phenomenon. This synchronization appeared to coincide with a reduction in tremor amplitude, indicating a potential causal relationship between stimulation-induced entrainment and tremor suppression.

The primary outcome of this study is the decrease in tremor amplitude observed when tSCS was administered at the frequency of the tremor. This reduction was evident throughout the entire 60-s trial compared with baseline but reached statistical significance only during the latter half of the stimulation period, indicating a buildup effect. Our results are in line with those observed in a previous study using phase-locked transcranial alternating current stimulation of the cerebellum.[18] In this article, the tremor amplitude significantly increased or decreased in the second half of the phase-locked stimulation, which in total lasted 30 s. However, differently from the present study, the stimulation at the tremor frequency (computed in real time) without phase-locking resulted in tremor amplitude reduction, albeit not statistically significant. This discrepancy could be related to the small sample size used in the previous study (11 subjects), but more importantly to the application of the alternating current stimulation on regions that have different implications in tremor generation. Using a computational model of the CTC network, the authors suggested that the suppression of tremor after cerebellar stimulation was due to a disruption of the temporal coherence of the aberrant oscillations in the olivocerebellar loop.[18] It is very likely that other mechanisms are implicated in the tremor changes observed in our study (see later), because the stimulation was applied at the level of the cervical spinal cord. Nevertheless, the findings from both studies clearly highlight the significance of stimulation frequency as a crucial parameter for modulating tremor. We showed, for instance, that the stimulation at 21 Hz, aligned with the corticospinal transmission in motor control,[30] did not alter tremor, while we observed a trend in tremor reduction only at 1 Hz. On the contrary, stimulation of the trapezius muscle increased tremor amplitude during the whole recording block, likely because of the propagation of the trapezius muscles' twitches induced by the stimulation in some subjects, as observed clinically. This negative result indicates that tremor suppression is not mediated by the recruitment of cutaneous afferent fibers. The absence of an effect from 21-Hz stimulation suggests a segregation between voluntary and involuntary activities, and that during tSCS physiologic movement-related firing rate is preserved, similarly to what it is observed with DBS.[31] Regarding 1-Hz stimulation, its typical action on inhibitory mechanisms remains consistent across various types of stimulation as evidenced by peripheral repetitive somatosensory stimulation,[32] repetitive transcranial magnetic stimulation,[33] or cellular studies[34] that reliably evoke long-term depression(-like) mechanisms when stimulation of around 1 Hz is applied. Therefore, in the present study, it can be assumed that low-frequency stimulation induced a nonspecific inhibitory effect, akin to the general effects observed with 1-Hz stimulation, leading to a mild reduction in tremor.

Tremor-Stim emerged as the most effective condition for tremor suppression, likely because of the timely disruption of neurogenic oscillations. Given that alternating current stimulation can entrain central neural populations,[35] this phenomenon likely contributed to the effectiveness of tSCS. Indeed, a third of the responders exhibited a high degree of tremor entrainment, correlating with a decrease in tremor amplitude. At first glance, this might surprise because tremor entrainment typically leads to an increase in amplitude, as observed in thalamic stimulation.[36] However, tremor phase–dependent modulation of brain alternating current stimulation does not always lead to changes of tremor amplitude,[37] and amplitude suppression is not always accompanied by a relevant effect on phase entrainment.[38] In addition, although the basal ganglia, motor cortex, and cerebellum might be differently implicated in the regulation of tremor amplitude and frequency,[39,40] in ET the modulation of descending oscillatory drive at the level of the spinal cord can regulate both, perhaps according to factors controlled by peripheral feedback or simply because of the direct involvement of motor neuron pools, which serve as the final common pathway for involuntary activity. This might indicate why tremor frequency significantly increased during Tremor-Stim, whereas tremor amplitude was suppressed, but it cannot be excluded that tremor frequency would increase as a consequence of the amplitude decrement.

The importance of the stimulus frequency in this context is supported by a study of epidural spinal cord stimulation investigating the frequency-dependence modulation of short- and long-latency EMG responses of lower-limb muscles in patients with spinal cord injury at rest.[27] The authors found that stimuli could evoke both types of response, but although the short-latency component was

enhanced at low frequencies and declined at higher rates, when eliciting a long-latency activity, the effect was more complex because the motor output could be expressed as suppression, tonic, or rhythmical activity. In addition, although rhythmical activity was sustained only with suprathreshold stimulation, the observation that its amplitude exceeded that of short- and long-latency responses suggests that additional variables, such as the central state of excitability, may be necessary to elicit rhythmic responses. This evidence provides insight into the entrainment phenomenon observed in our patients, indicating that it may occur under specific circumstances.[27]

Considering the novel application and the limited knowledge about how tSCS activates spinal neurons, the mechanisms underlying tremor suppression induced by tSCS can only be inferred. In general, it has been shown that tSCS is able to increase excitability of local spinal networks via dorsal root afferents recruitment.[26,27] We can therefore assume that the activation of the dorsal root with a short pulse of stimulation would be followed by a period of suppression and, because the tremor is not coherent with the tSCS, tremor and dorsal root inputs would oppose each other, resulting in tremor reduction. A similar mechanism is postulated for tremor suppression during peripheral stimulation; however, tSCS could be more effective because of the anatomical separation from motor fibers and the stimulation of a larger number of afferents compared with peripheral nerve stimulation. At higher frequency (21 Hz), the periods of suppression may be less powerful and abruptly ended by excitation from subsequent stimuli, resulting in no tremor changes. In addition, the activation of large–medium afferent fibers in the posterior root might disrupt tremorgenic circuits through the afferent pathways projecting into the tremor oscillatory network. This could be either via the thalamus or the cerebellum, as proposed by other studies with peripheral and brain stimulation.[29] However, cervical tSCS can also alter the motor output by engaging sensory pathways that transsynaptically converge on motor pools projecting to upper limb muscles.[41] In light of this, the effect of tSCS on tremor could be attributed to the modulation of spinal reflexes, because group Ia afferents are known to contribute to tremor amplification through monosynaptic reciprocal inhibition.[42] If this is the case, the simultaneous activation of agonist and antagonist afferents may reduce the alternating muscle activity characteristic of tremor, thereby suppressing it.

Interestingly, recurrent inhibition via Renshaw cells in the spinal cord has been proposed as a possible "neural filter" that leads to partial cancellation of brain oscillations less than 10 Hz.[43] According to this study, the Renshaw cell feedback loop plays an important role in reducing 10-Hz oscillations in muscle, which aligns with the typical frequency of physiologic tremor. Moreover, Renshaw cells can also filter lower frequencies and it has been postulated that strengthening of Renshaw cell feedback could lead to reduction in tremor amplitude, presumably via indirect circuits.[43] Hence it is reasonable to speculate that these mechanisms could have an important role in ET, and that the tSCS protocol used in our experiment could modulate these spinal circuits, limiting the transmission of tremor oscillations to the muscles. An interplay between spinal and supraspinal effects cannot be discarded because of the nature of the stimulation, because brain circuits are engaged through afferent fiber projections, which modulate the motor output at the spinal cord level. Further studies will be required to unravel the circuits modulated by cervical tSCS in tremor.

We did not find any relevant linear relationships between the tremor's features and the stimulation effects; therefore, a tremor responsiveness profile could not be identified. However, for the Tremor-Stim condition, there was a significant positive correlation between the baseline tremor frequency and the change in tremor frequency, ie, higher-frequency tremor is more sensitive to an increase in tremor frequency during Tremor-Stim, a finding that will be interesting to explore further.

Spinal cord electrical stimulation has been previously tested to manage different types of tremor. For instance, a recent study showed that trans-spinal direct current stimulation (using a constant current instead of a rhythmic stimulation) could ameliorate instability in primary orthostatic tremor.[44] Epidural or thoracic spinal cord stimulation has also been tested in patients with Parkinson's disease, and preliminary results suggested an overall improvement of symptoms in this population.[45] Although these modalities of electrical stimulation differ from those used in our study, they share mechanisms linked to the modulation of synaptic efficacy at the dorsal horn level, with the potential influence at supraspinal levels via ascending spinal pathways.

Some limitations of the study should be acknowledged. These include the relatively small number of subjects and inclusion of both ET and ET plus patients with slightly different clinical features. The latter reflects the heterogeneity of this syndrome, which is unlikely to be relevant in the study outcome because our method acted on the transmission of oscillations through the spinal cord and not the brain pathophysiologic source. Furthermore, closed-loop stimulation phase-locked to the tremor, as implemented in previous studies, was not employed, because our primary objective was to examine the potential impact of different stimulation frequencies on modulating spinal cord activity. For the same reason, the study was not designed as a clinical trial.

In conclusion, our study demonstrated that open-loop cervical tSCS can effectively reduce postural tremor in ET when delivered at the subject's tremor frequency. We postulated that the changes in tremor amplitude are induced by the modulation of the spinal cord circuits

by tSCS, which possibly disrupts the oscillatory drive to muscles by acting on afferent pathways or spinal reflexes; however, we acknowledge that there may be an interplay between spinal and supraspinal centers contributing to this effect.

This study represents an initial step in understanding the potential of tSCS to interact with ongoing neural rhythms in the central nervous system. Our findings indicate that tSCS may be a promising approach for targeting pathologic tremors. Based on previous studies, we assume that prolonged stimulation might result in sustained after-stimulation effect,[18,44] but it is essential to ascertain whether the capability of tSCS in disrupting tremor depends on continuous application or if its effects persist after the stimulation has ceased. In addition, it should be explored how tSCS interacts with tremors across various motor tasks, including dynamic activities. Finally, further research is warranted to fully elucidate the mechanisms underlying tSCS-induced tremor suppression, the observed increase in tremor frequency, and the high level of entrainment observed during effective stimulation. Such investigations will contribute to a better understanding of the therapeutic potential of tSCS in managing ET and to the development of optimized stimulation protocols tailored to individual patient characteristics. ■

Data Availability Statement

The data that support the findings of this study are available from the corresponding author, 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.

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Supplementary material.

Experimental design

Participants comfortably sat on a chair during the experiment. For the tSCS protocols, the stimulation electrode serving as cathode (3.2 cm diameter, Axelgaard Pals Platinum) was placed between C5 and C6 spinous processes, while two stimulation electrodes (4x9 cm, Axelgaard Pals Platinum) serving as anode were placed over each clavicle. For the trapezius muscle stimulation (Trap-Stim) condition (see below), the cathode electrode was placed over the upper trapezius muscle. TSCS stimulation was delivered with a biphasic constant current stimulator (DS8R, Digitimer), using a 5 kHz carrier frequency with biphasic and symmetric rectangular pulses (90 us pulse width) [28], at different stimulation frequencies: tremor frequency (Tremor-Stim), 1 Hz and 21 Hz. One Hz stimulation aims at disrupting the oscillatory tremor input as proposed by other studies using non-invasive brain or peripheral stimulation [29, 30]; 21 Hz stimulation was used with the intent to target the excitability of afferent interneurons located in the dorsal horns [27], while 21 Hz stimulation over the trapezius muscle (Trap-Stim) ipsilateral to the selected hand served as a control, since stimulation over the muscle belly would mainly recruit cutaneous afferent fibres rather than the dorsal roots [31]. In summary, 5 conditions were tested while the subjects hold a posture: (1) no stimulation (referred to as Baseline), (2) Tremor-Stim; (3) 1Hz; (4) 21 Hz and (5) Trap-Stim.

Tremor was recorded at Baseline and during each stimulation by a triaxial accelerometer (*MXR9500G/M, Memsic*) placed on the dorsum of the hand most affected by tremor. Acceleration signals were digitized at 5000 Hz with a data acquisition board (DAQ, model Power *1401*, Cambridge Electronic Design Limited, Cambridge, UK) controlled with *Spike2* (developed by the same company).

To determine the stimulation intensity and confirm the site of stimulation, recruitment of muscle activation was measured. To this aim, we delivered 10 tSCS stimuli at 1 Hz for each stimulation intensity, from 50 mA to 100 mA, at 10 mA increments. Muscle responses were recorded by surface bipolar electrodes placed over the first dorsal interosseus (FDI), flexor carpi radialis (FCR), biceps brachii and deltoid of the tested arm, to prove that all the muscle potentially involved in the tremor were activated by the stimulation. Signals were bandpass filtered (5 Hz - 2 kHz) with a Digitimer D360 (Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK) and digitized at 5 kHz with the DAQ mentioned before. The motor threshold (MT) was defined as the intensity to obtain a 50-100 μV electromyographic (EMG)

response on the FCR (the most involved in ET). Stimulation intensity for all the conditions was set below the MT (minus 10 mA) to avoid recruitment of motoneurons and interference with motor control of the upper limbs. Exceptions included participants who exhibited limited muscle activity at 100 mA or that could not tolerate a sufficient intensity. For those patients, the stimulation intensity was set as the maximum intensity tolerated (always below the MT).

To determine the tremor frequency, we estimated the power spectral density (PSD) of the accelerometer data while patients held the arms outstretched during 60 s. The PSD was estimated using the *Power Spectrum* function of *Spike2* (window length 6.554s, 50% overlapping, frequency resolution 0.1526 Hz) and the tremor frequency determined between 3 Hz and 9 Hz band.

The experiment consisted of 60 s tremor recording (block), during which patients had to hold their arms outstretched and pronated, at each stimulation condition (Baseline, Tremor-Stim, 1 Hz, 21 Hz or Trap-Stim). Each condition was repeated four times and the order of the blocks was randomized. The participants were blinded to the stimulation condition and consecutive blocks were separated by 60 s resting periods without stimulation.

Data analysis and statistics

Acceleration in the z-axis (wrist flexion-extension) was used to estimate the tremor amplitude [32, 33]. To do this, the recorded signals were filtered in the tremor frequency band (3-9 Hz) using a 2nd order zero-lag Butterworth filter. The instantaneous amplitude of the tremor signal from the accelerometer was computed by obtaining the absolute value of the Hilbert transform. Then, the signal was split into 1-s epochs and the tremor amplitude for each epoch was estimated computing the mean of the instantaneous tremor amplitude. To normalize the recorded epoch tremor amplitudes, they were z-scored using the mean and standard deviation of the tremor amplitudes obtained in the Baseline blocks.

The tremor frequency was also estimated for each 1-s epoch within a block. This was done by computing the power spectral density using Welch's method (1-s windows) and identifying the frequency at which the maximum power was found between 3 Hz and 9 Hz. Similarly to amplitudes, z-score normalization was used for the estimated frequencies. Statistics for tremor amplitude and frequency were computed in three periods: the entire block (60 s), the first half of the block (1-30s), and the second half of the block (31-60s). This windowing analysis method was based on results showed by previous studies and on the trends

detected in the data (see below). Extreme outliers' blocks were removed from the analysis using the Grubbs test (6 out of 357 blocks) [34]. In addition to the z-scores, results were reported as a relative reduction of tremor as a percent of baseline (the baseline tremor amplitude or frequency were subtracted from those of the different tSCS conditions and divided by average baseline tremor). Linear-mixed model analysis was used to explore the differences in tremor amplitude and frequency (z-scores) at the group level elicited by the different stimulation protocols, using the stimulation condition as fixed effects, and the subject as random effect. P-values were computed using post-hoc tests with Bonferroni correction for multiple comparisons (*i.e.*, differences were considered significant for equivalent *p-values* below 0.005).

To investigate the subject-specific responsiveness to the stimulation protocols, Wilcoxon-ranked tests with Bonferroni correction were used to analyse tremor amplitude and frequency across the 1-s segments across stimulation conditions (Baseline, Tremor-Stim, 1 Hz, 21 Hz and Trap-Stim) since normality assumption of the data was not satisfied [18].

We also analysed the synchronization between the tremor phase and the phase of the stimulation in the Tremor-Stim blocks. The phase difference between the acceleration and the modulating signal used to generate the stimulation was computed for this purpose. Both signals were band-pass filtered around the estimated mean tremor frequency in the block using a 2-Hz band-pass filter (2^{nd} order zero-lag Butterworth filter). Then, the instantaneous phases of both signals were estimated from the Hilbert transform [35]. To characterize the phase-locking between the two signals during periods when tremor was present, a heuristic threshold was used to discard periods of the signal with insufficient levels of tremor. The phase difference between the two signals was computed by subtracting phase values and the mean phase difference was computed using the circular mean. The Phase Locking Value (PLV) was used to estimate the synchronization between the two signals. PLV is a common non-directional connectivity measure of the phase relationship of two signals, it ranges from 0 to 1 and it is independent of the signal amplitudes [36]. To determine a threshold above which PLV levels should be considered significant, we computed the PLV levels between the tremor signals measured during the baseline blocks and a simulated cosine function at the tremor frequencies. The statistical threshold to determine significant PLV levels for each subject was defined as the mean value of the PLV levels obtained from the baseline blocks plus 3 standard deviations across subjects. Circular correlation was used to study the relationship between the mean phase difference and the PLV.

Finally, to characterize the relationship between the effects of stimulation and tremor features, the Pearson's correlation coefficient was calculated between the following variables across conditions: z-scores estimating change in tremor amplitude and frequency, absolute values of tremor amplitude and frequency at baseline, PLV during Tremor-Stim, age, FTM scores, MT and stimulation intensity.

Results

The results are detailed in the Supplementary Materials. Eighteen patients completed the study. One subject was excluded from the analysis because the experimental task was not completed due to fatigue induced by prolonged and repeated maintenance of an outstretched arm posture. Their data were unusable for analysis due to incompleteness. No adverse effects of stimulation were reported by any of the patients. However, in some cases (as indicated in Table 1) stimulation intensity was lowered below the usual intensity of MT minus 10 mA because of pain, particularly at 21 Hz stimulation. Clinical features and stimulation parameters are summarised in Table 1.

Fig. 2A shows the evolution of the tremor amplitude (average across subjects) during the different stimulation blocks. When considering the entire stimulation period of 60 s, Tremor-Stim condition resulted in a reduction of tremor amplitude (z-score = -0.33 ± 0.90; -13 ± 42 % tremor reduction), although the difference with the Baseline conditions was not significant (Fig. 2B, E). Tremor amplitude in Trap-Stim blocks (z-score = 0.55 ± 1.56; 31 ± 91 % tremor aggravation) was significantly higher than in Tremor-Stim (z-score = -0.33 ± 0.90, p = 0.001) and 1 Hz conditions (z-score = -0.24 ± 0.83, p = 0.013; 4 ± 41 % tremor reduction).

To account for potential build up effects in tremor modulation (in line with previous similar studies [18] and compatible with the trends displayed in Fig 2A), we also analysed separately the changes in tremor amplitude during the first and second 30 s periods of each block. During the first 30 s (Fig. 2C, F), Trap-Stim resulted in a significantly higher tremor amplitude (z-score = 0.741 ± 1.88; 50 ± 134 % tremor aggravation) than 1 Hz stimulation (z-score = -0.153 ± 0.90, p = 0.022; 2 ± 43 % tremor aggravation). In the second 30 s period of the stimulation, Tremor-Stim resulted in a significant tremor reduction (z-score = -0.63 ± 0.90; -25 ± 35 % tremor reduction) compared to Baseline (z-score = 0.05 ± 0.31, p = 0.005), Trap-Stim (z-score = 0.35 ± 1.34, p <0.001; 18 ± 65 % tremor aggravation) and 21 Hz (z-score = -0.05 ± 0.80, p = 0.024; -4 ± 29 % tremor reduction) conditions (Fig. 2D, G). This result can be observed in Fig. 2A, where the z-scores of the tremor amplitude are markedly reduced after

30-s of stimulation delivered in the Tremor-Stim condition. TSCS applied at 1 Hz resulted in a non-significant tremor reduction relative to baseline (z-score = -0.33 ± 0.85; 7 ± 45 % tremor reduction) in the last 30 s period.

Regarding the stimulation effects on tremor frequency, the Tremor-Stim condition elicited a statistically significant increase in the tremor frequency in the 60 s window (z-score = 0.29 ± 0.88; 4 ± 12 % tremor frequency increase) compared to Baseline ($p = 0.013$) and 21 Hz conditions (z-score = -0.02 ± 0.29, $p = 0.005$; 0 ± 3 % tremor frequency change) (Fig. 3A, B, E). A significant increase in tremor frequency was also found during Tremor-Stim for the first 30 s period of stimulation (z-score = 0.38 ±0.92; 3 ± 12 % tremor frequency increase) compared to Baseline ($p = 0.046$) and 21 Hz stimulation conditions (z-score = 0.11 ± 0.32, $p = 0.041$; 0 ± 3 % tremor frequency change) (Fig. 3C, F). Similar results were obtained during the last 30 s period, with a significant increase in the tremor frequency during the Tremor-Stim condition (z-score = -0.20 ± 0.82; 4 ± 12 % tremor frequency increase) compared to Baseline ($p = 0.023$) and 21 Hz stimulation (z-score = -0.16 ± 0.29, $p = 0.004$; 1 ± 3 % tremor frequency change) (Fig. 3D, G).

Patient-specific responsiveness to the different stimulation conditions was also analysed [18]. Z-scores of tremor amplitude computed from the second half of the stimulation blocks were compared against the Baseline condition. According to their response to the stimulation, patients were assigned to one of three possible groups: positive responders (tremor amplitude decreased statistically significantly during stimulation), negative responders (tremor amplitude increased statistically significantly) and neutral responders (tremor amplitude was left unchanged). Tremor-Stim resulted in the highest number of positive responders (11 subjects), with 3 subjects identified as negative responders and 4 subjects as neutral responders (Fig. 2I). Fig. 2H displays the z-scores averaged values across the 60 s blocks and subjects grouped by the responsiveness to the stimulation phase-locked to the tremor frequency.

The phase difference between the accelerometric signal and the stimulus was computed across the Tremor-Stim blocks for all the subjects (Fig. 4A). Stimulation data for subjects 1 and 3 were missing due to a technical problem (the stimulus signal was not stored). Five out of 16 subjects showed statistically significant PLVs, meaning the tremor phase was consistently synchronized with the delivered stimulation (Fig. 4B). We did not find any relationship between the mean phase difference and the strength of the synchronization (PLV) at the group level (circular correlation = 0.21, $p = 0.69$), nor at the sub-group level considering only subjects

showing significant synchronization (circular correlation = 0.87, $p = 0.14$). Interestingly, higher levels of PLV were associated with tremor reduction: in 4 out of the 5 subjects showing a significant phase locking with the stimulation, the average tremor amplitude measured during the Tremor-Stim blocks was smaller than the tremor in the baseline blocks (Fig. 4C). This means that when tremor was successfully entrained by stimulation, its amplitude decreased compared to baseline.

Finally, to explore potential relationships between the effects of tSCS and tremor features, linear correlation analysis was performed between pairs of variables across the different stimulation conditions (Fig. 5). A positive linear relationship (rho = 0.62) between the tremor amplitude and the FTM scores was also present in the study. For the Tremor-Stim condition, statistically significant correlation was found between the subject baseline tremor frequency and the change in tremor frequency (rho = 0.56). This result indicates that a higher-frequency tremor tends to be more sensitive to an increase in tremor frequency during Tremor-Stim. For the 1 Hz stimulation condition, a negative relationship (rho = 0.68) between the subject baseline tremor amplitude and the effect in tremor amplitude was reported, meaning the higher the tremor severity, the higher the tremor reduction effect.

No influence of chronic Botulinum toxin treatment on tSCS responsiveness was observed in this study since 3 out of 7 patients receiving Botulinum toxin were responsive to the Tremor-Stim tSCS protocol. Finally, regarding the specific syndromic diagnosis, 5 out of 7 patients (71%) diagnosed with ET were responsive to the Tremor-Stim tSCS protocol (2 non-responders), while 6 out of 11 patients (55 %) with ET plus positively responded to the Tremor-Stim tSCS protocol (2 non-responders, 3 negative responders). Thus, there was no difference in the response between the two groups of patients.