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Effect of short-term transcutaneous trigeminal nerve stimulation on EEG activity in drug-resistant epilepsy

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ABSTRACT

Background: Transcutaneous trigeminal nerve stimulation (TNS) has antiepileptic effects in patients with drug-resistant epilepsy (DRE). However, whether and how TNS is able to modulate the electroencephalogram (EEG) background activity in patients with DRE is still unknown.
Objectives: To investigate the effect of short-term TNS on EEG background activity in DRE by qualitative and quantitative analyses.
Methods: Twenty-nine DRE patients participated in the study. Twenty-two were randomly divided into a "sham-TNS" or "real-TNS" group; seven patients underwent stimulation of the median nerve (MNS) at the wrist. Real-TNS was delivered bilaterally to the infraorbital nerve (trains of 1–20 mA, 120 Hz, cyclic modality for 20 min). The sham-TNS protocol mimicked the real-TNS one but at a zero intensity. For MNS, the same parameters as real-TNS were used. EEG was continuously acquired for 40 min: 10′ *pre*, 20′ *during* and 10′ *post* stimulation. EEG was visually inspected for interictal epileptiform discharge (IEDs) changes and processed by spectral analysis for changes in mean frequency and absolute power of each frequency band.
Results: A significant increase of EEG absolute alpha power was observed *during* real-TNS compared with the sham-TNS ($F_{34,680} = 1.748$; $p = 0.006$). Conversely, no significant effects were noticed either for quantitative analysis of other frequency bands or for IEDs detection. MNS proved unable to modulate EEG activity.
Conclusions: Short-term TNS induces an acute and specific effect on background EEG of DRE by increasing the absolute alpha band power. EEG alpha rhythm enhancement may index a cortical functional inhibition and act as a seizure-preventing mechanism.

1. Introduction

Drug-resistant epilepsy (DRE) accounts for 30% of the worldwide epileptic patients for whom, despite adequate pharmacological therapies, seizures are not completely controlled [1]. Excluding a small percentage of DRE patients eventually suitable for curative epilepsy surgery, most of them continue to have lifelong disabling seizures with a significant burden on quality of life [2,3].

Considering the increasing need for novel therapeutic options for DRE and historical observations that electrical stimulation of subcortical structures could modify cortical excitability [4–6], a number of neuromodulation devices have been developed over the past few decades.

Vagal nerve stimulation (VNS) represents the most widely used neurostimulation method, being the only peripheral approach currently approved by the US Food and Drug Administration (FDA) as an adjunctive treatment for partial-onset DRE. It shows a 1-year responder rate (≥50% reduction in seizure frequency) by up to 36.8% of the treated patients [7,8].

Moreover, in the last decade a large body of evidence has supported the use of an alternative peripheral neuromodulation method, named trigeminal nerve stimulation (TNS). TNS has recently received European Conformity (CE) marking as a promising additional treatment for intractable epilepsy [9].

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Table 1
Characteristics of epileptic patients.

Experimental Group	Case	Sex	Age (years)	Type of Epilepsy	Lesion	N of seizures (/month)	AEDs
TNS SHAM	1	F	52	RFr	Malacic	7	LEV + LTG
	2	M	65	LT	HS	2	VPA + PER + PRI + LEV
	3	M	67	LFr	Schizencephaly	3	OXC + LEV + PGB
	4	M	44	LO	Gliosis	2	PER + LTG + LAC
	5	M	61	RT	HSE	15	LAC + LEV + CBZ
	6	M	42	LFr	Malacic	15	VPA + LTG + TPM
	7	M	53	RT	Crypto	7	CBZ + LAC + VPA + LEV
	8	F	54	RP	SAH	8	CBZ + LEV
	9	F	46	RT	Crypto	8	LTG
	10	F	66	LT	Crypto	18	LEV + LTG
	11	M	35	LT	Crypto	35	LEV + LTG
TNS REAL	1	M	31	LFr	Crypto	2	CBZ + LTG
	2	M	65	LT	Crypto	2	CBZ
	3	F	27	RT	HS	4	LEV + LAC
	4	M	68	BFr	Crypto	2	LEV + PB + CBZ
	5	M	49	RFr	RE	3	LEV + CBZ
	6	F	37	RO	Crypto	2	TPM
	7	F	31	RFr	Malacic	10	VPA + LTG + LAC
	8	F	57	LT	Crypto	15	CBZ + ZNS
	9	F	51	RT	Crypto	17	CBZ + LEV
	10	F	35	BT	NH	3	LEV + LTG + LAC
	11	F	37	RT	FCD	40	LEV
MNS	1	F	21	LFr	Gliosis	2	LEV + VPA
	2	M	68	RT	Crypto	12	CBZ
	3	F	60	LP	Cavernoma	4	LEV + TPM
	4	F	22	RP	Crypto	20	CBZ + LEV
	5	M	69	LT	Crypto	15	VPA + LEV
	6	F	31	RT	NH	2	LTG
	7	F	67	LFr	Gliosis	6	VPA + LEV + LTG

TNS, trigeminal nerve stimulation; MNS, median nerve stimulation, F, female; M, male; R, right; L, left; B, bilateral; Fr, frontal; T, temporal; P, parietal; O, occipital; Crypto, cryptogenic; FCD, focal cortical dysplasia; HS, hippocampal sclerosis; HSE, Herpes simplex encephalitis; NH, nodular heterotopia; RE, Rasmussen encephalitis, SAH, subarachnoid hemorrhage; CBZ, carbamazepine; LAC, lacosamide; LEV, levetiracetam; LTG, Lamotrigine; OXC, oxcarbazepine; PB, phenobarbital; PER, perampanel; PGB, pregabalin; PRI, primidone; TPM, topiramate; VPA, valproic acid; ZNS, zonisamide.

This method seems to have some advantages in comparison with the well acknowledged VNS. Indeed, while VNS implantation represents an invasive procedure, carrying the risk of surgical intervention and several adverse effects [10,11], TNS is an external peripheral method which can be safely used and easily self-administered by the patients [12]. Moreover, the absence of autonomic fibers in the trigeminal nerve allows bilateral stimulation with larger effects at lower intensities compared with unilateral stimulation, as routinely used for VNS [13,14].

Despite their wide clinical utilization, the mechanisms underlying the anticonvulsant effect of cranial nerves stimulation are not completely understood.

Animal models of epilepsy suggested that VNS may modulate cerebral activity by inducing an electroencephalographic (EEG) desynchronization and so a cortical inhibition, ultimately leading to an increased electric threshold at the epileptogenic tissue [15,16]. However, human studies focused on the impact of VNS on EEG activity of epileptic patients did not reach unequivocal conclusions, showing different and sometimes contradictory results such as synchronisation or desynchronization effects on different frequency bands or changes in EEG frequency power [17–22].

As well as VNS, TNS has been shown also to influence cortical activity in a single, pioneering animal model, by means of a thalamocortical desynchronization effect [14].

Trigeminal and vagus nerves share widespread projections to different subcortical structures with cortical neuromodulatory effects; therefore they were also supposed to share some anticonvulsant mechanisms [14,23–26].

However, whether and how TNS is able to modulate human cortical excitability and background EEG dynamics in DRE patients is still unknown.

Recently, some neurophysiology works in healthy subjects provided evidence regarding TNS-induced brainstem modulation [24] and brainstem plasticity in a long-term depression manner [26]. By contrast, primary motor cortex (M1) excitability and sensorimotor integration at cortical level appeared unaffected by acute stimulation, suggesting no effect of TNS on M1 [24,27].

Contrariwise, a frequency-dependent inhibition of neuronal firing in primary somatosensory cortex (SI) neurons, in response to contralateral TNS, has been recently shown in rat models [28], in accordance with a previous work showing EEG spike suppression in SI and thalamus after TNS delivery [14].

Therefore, while it seems clear that subcortical structures play a fundamental role in seizure regulation mechanisms by TNS [23–26], evidence regarding the TNS capacity to alter cortical excitability and/or EEG dynamics is scarce and inconclusive [14,24,27,28] and cannot be easily translated to DRE patients as an explanation of TNS clinical benefits.

TNS effects on background EEG activity were neither proved nor investigated in these patients. Therefore, with this study, we aimed to evaluate the effect of an acute TNS protocol on cortical EEG background activity in DRE patients, by conducting a qualitative (morphologic) and quantitative (power spectra) EEG analysis in an active vs a sham-TNS group. Specificity of a possible TNS effect was also investigated by examining a control group undergoing median nerve stimulation (MNS).

2. Material and methods

2.1. Subjects

Twenty-nine subjects affected by DRE (16 females and 13 males; 48.66 ± 15.45 years old; range 21–69 years), who attended the Center for Diagnosis and Treatment of Epilepsy at the Unit of Neurology, University of Sassari (Italy), were recruited for the study. They were selected based on the following inclusion criteria: age 18–70 years; diagnosis of focal DRE [1] with ≥2 partial or secondary generalized tonic-clonic seizures (GTC) per month for the last 2 consecutive months and concurrent use of ≥1 antiepileptic drug (AED). Exclusion criteria included history of diabetes, migraine or trigeminal neuralgia; presence of cardiovascular diseases (such as arrhythmia, heart failure or ischemic heart disease) or other serious medical or psychiatric conditions and ongoing pregnancy. Characteristics of selected patients are summarized in Table 1.

The experimental procedure was approved by the local ethical committee (Bioethics Committee of ASL n.1 Sassari, Prot n. 982/CE) and conducted in accordance with the Helsinki Declaration. An informed written consent was obtained from all the subjects before conducting the study. According to previous clinical studies performed in DRE patients [29], 22 out of 29 patients were divided into two groups "sham-"or "real-TNS": after baseline evaluation, 22 opaque envelopes were numbered consecutively and randomly assigned to an intervention (real-TNS; $n = 11$) or to a no-intervention (sham-TNS; n = 11) group, with a blocking procedure employing the Research Randomizer 3.0 software. Both subject and statistician were blinded for the type of intervention. To investigate the specificity of TNS effect, 7 patients naïve to the main experiment participated in a control experiment where bilateral and unilateral MNS ("bil-MNS and "uni-MNS", respectively) were delivered.

2.2. EEG recording

EEG signals were recorded with head caps using a 19 channel EEG system (Brain Quick System, Micromed, Mogliano-Veneto, Italy) in physical reference with successive reconstructions of bipolar derivations (FP1-F3, F3-C3, C3-P3, P3-O1, FP1-F7,F7-T3,T3-T5,T5-O1,FP2-F4,F4-C4,C4-P4,P4-O2,FP2-F8,F8-T4,T4-T6,T6-O2,FZ-CZ,CZ-PZ) according to the international 10–20 system. EEG was acquired in eyes-closed resting state, band pass filtered between 0.5 and 70 Hz and digitized with sample frequency set at 256 Hz. Patients were asked to maintain vigilance throughout the recording. Vigilance was evaluated continuously by experienced clinical physiologist on the basis of the EEG signal variation. EEG was continuously recorded for a 40-minute period comprising 3 phases: (i) 10 min of baseline EEG, recorded before stimulation (pre) (ii) 20 min during the intervention (real- or sham-TNS and bil- or uni-MNS) (iii) 10 min after intervention (post). Electrocardiogram and electrooculogram (EOG) were simultaneously monitored in all the subjects (time constant: 0.3 s, filter 30 Hz). EOG was recorded from the orbicularis oculi muscle by surface electrodes, with the active electrode placed over the mid lower eyelid and the reference, 2-3 cm lateral.

2.3. TNS

Real-TNS was delivered bilaterally to the infraorbital nerve (ION) through 26-mm-diameter disposable, hypoallergenic, silver-gel self-adhesive stimulating electrodes (Globus, Domino s.r.l., Codognè, TV, IT) placed over the ION foramina and connected to a Winner® stimulator (Fisioline biomedical instrumentation, Verduno, CN, IT). The stimulus consisted of trains of a symmetric biphasic square wave pulse (duration 0.25 ms, frequency 120 Hz), delivered in a cyclic modality (30 s ON and 30 s OFF) for 20 min, according to previous works [24,26,30,31]. Stimulation intensities ranged from 1 to 20 mA and corresponded, for each ION, to the maximal pain sub-threshold intensity endurable comfortably by the subject (10.52 ± 1.72 mA).

The sham-TNS protocol mimicked the initial bilateral real-TNS stimulation and consisted of a calculation of both perceptual and pain threshold (11.62 ± 1.69 mA), followed by 20s of TNS, the intensity of which was subsequently gradually decreased down to zero, which corresponded to the OFF position of the stimulator. Before the experiments began, patients were told that they could receive a stimulation with different intensity and/or frequency and that they could perceive very light stimulation or very high but that it was anyway lower than pain threshold. Moreover the operator explained to the patients that it could be possible to habituate to the stimulus so that they could no longer perceive it.

2.4. MNS

MNS was delivered through 26-mm-diameter disposable, hypoallergenic, silver-gel self-adhesive stimulating electrodes (Globus, Domino s.r.l., Codognè, TV, IT) placed over the wrist bilaterally (bil-MNS) or contralaterally to the epileptic focus (uni-MNS). The same stimulation parameters used for real-TNS were employed in this control experiments, which were carried out in two sessions separated by at least one week.

2.5. EEG analysis

EEG recordings were carried out by an experienced clinical neurophysiologist and visually analyzed independently by two epileptologists.

Artifacts due to eye blinks, horizontal–vertical eye movements and signs of sleepiness were detected by visual inspection and deleted. Sleepiness was defined as the appearance of electrophysiological markers of drowsiness or any unequivocal sleep stage within a 30-sec epoch, according to the AASM "Manual for the Scoring of Sleep and Associated Events' [32]. In order to rule out drowsiness as a confounding factor for the analysis, its presence was assessed both in terms of raw value (absolute number) and density (i.e. number of drowsiness moments /min of EEG recording) for each EEG phase (pre, during and post-stimulation).

The EEG recordings were visually analyzed to identify epileptic waveforms. Only definite spikes, sharp waves, and spike-wave complexes were considered epileptiform abnormalities and distinguished from the background activity due to their morphology and/or amplitude. Only the epileptic graphoelements that both observers agreed on were included in the analysis.

Two different paradigms were considered: the raw value (absolute number) of interictal epileptiform discharges (IEDs) and the IEDs density (i.e. number of IEDs/minutes of EEG recording) for each EEG phase (pre, during and post-TNS). Both calculations were included in the statistical analysis.

Bipolar EEG signals of each recording were processed by spectral analysis: Fast Fourier Transform was applied to 4-sec artifact-free basic epochs and mean spectra were computed according to the Welch's method (with Tukey window) for each phase (pre, during and post). Due to the overlapping of the stimulation artifact with EEG signals, the analysis of data collected during TNS (real and sham) or MNS (bilateral and unilateral) was limited to the OFF phase only, with the exclusion of the 3-second periods at the beginning and end. To assess the timing of the possible stimulation effect of real-TNS, the individual OFF periods ($n = 20$) comprised in the during period were considered for further analysis.

Based on mean spectra, absolute power was computed for each frequency band: delta (0.5–3 Hz), theta (3.1–7.0 Hz), alpha (7.1–13 Hz), beta (13.1–30 Hz) and gamma (30–48 Hz). Power-weighted mean frequency was computed in a large band (0.5–48 Hz) in order to measure a possible global frequency shift.

2.6. Statistical analysis

Comparison of demographic and clinical variables between groups (real- or sham-TNS; bil- or uni-MNS) was performed with parametric one-way ANOVA, with Group as a between factor, for continuous variables and Pearson's $\chi 2$ test for categorical variables.

To assess the effect of the intervention on qualitative EEG variables (IEDs) and possible confounding factor (drowsiness), a two-way repeated-measures (RM) ANOVA was used with TIME (pre-, during- and post- intervention) as within factor and CONDITION (sham- or real-TNS, bil- or uni-MNS) as between factor.

In order to compare real and sham TNS effect on quantitative EEG variables, such as mean frequency and absolute power of each frequency band, an RM-ANOVA was used with CONDITION (sham- and real-TNS) as between subjects factor and TIME (pre-, during- and post-TNS), and DERIVATION (spatial position) as within subject factors.

Given the large number of comparisons, values were corrected for multiple comparisons using the false discovery rate (FDR) method [33] which is less conservative than Bonferroni correction, and better suitable for assessing global variations.

Moreover, in order to investigate specificity of TNS effect, the same approach was used to analyze data from the MNS group by a three-way within subject RM-ANOVA with TIME (pre-, during- and post-intervention), CONDITION (bil- or uni-MNS) and DERIVATION (spatial position) as factors.

To identify a possible timing effect of the real-TNS an explorative post-hoc statistical analysis was also conducted. Indeed the quantitative EEG variable affected by TNS was further analyzed by RM-ANOVA considering TIME (from the 1st to the 20th interval in the During phase) as within subjects factor.

Statistical analysis was performed using the SPSS 18 software (SPSS Inc., Chicago, IL, USA).

3. Results

No significant difference in any of the demographic and clinical features (age $F_{2,26} = 0.892$; $p = 0.42$; sex $p = 0.27$; number of seizures $F_{2,26} = 0.136$; $p = 0.87$; number of AEDs $F_{2,26} = 2.507$; $p = 0.10$) were found between groups.

Qualitative analysis of IEDs in terms of raw values and density showed no significant effect for TIME ($F_{2,31} = 1.189$, $p = 0.30$; $F_{2,31} = 0.203$, $p = 0.80$, respectively), CONDITION ($F_{3,32} = 1.847$, $p = 0.15$; $F_{3,32} = 2.100$, $p = 0.11$, respectively) or interaction among factors.

Statistical analysis of drowsiness detected no significant effects of TIME for both raw values and density ($F_{2,31} = 0.023$, $p = 0.98$; $F_{2,31} = 0.535$, $p = 0.58$, respectively), CONDITION ($F_{3,32} = 0.770$, $p = 0.55$; $F_{3,32} = 0.762$, $p = 0.56$, respectively) or interaction among factors.

RM ANOVA for quantitative EEG variables showed a significant effect of DERIVATION, which was observed for all variables. Absolute power of each frequency band was not significantly affected by TIME and CONDITION and no significant interactions among the factors were observed for any variable, except for the absolute power of alpha band (Table 2).

A significant increase in alpha power in the real-TNS group during stimulation was detected, with a greater effect on posterior areas (Fig. 1) and with a significant effect for TIME and for the interactions DERIVATION*TIME and DERIVATION*TIME*CONDITION (in real-TNS condition Pre vs During: C3 $p = 0.0095$, P3 $p = 0.0001$, O1 $p = 0.0001$, P4 $p = 0.0159$, O2 $p = 0.0001$, Pz $p = 0.0021$, *T5* $p = 0.0004$, T6 $p = 0.001$) (Table 2). The statistical analysis showed no difference between the Pre and the Post.

A post-hoc analysis explored the trend of alpha power in the posterior regions across successive intervals in the During phase. ANOVA for repeated measures showed a significant effect of TIME (successive intervals, $F_{18,126} = 2.28$, $p < 0.01$). Post Hoc analysis showed that the TNS effect on mean alpha power was significant at the 4th cycle of stimulation (i.e. after 4 min from the beginning of TNS), then exhibited a gradual decrease with return to baseline after the 14th cycle of stimulation (i.e. after 15 min of stimulation).

The statistical analysis of the control experiment showed a significant effect, for all quantitative EEG variables, for DERIVATION. By contrast, absolute power of each frequency band was not significantly affected by TIME and CONDITION and no significant interactions among the factors were observed.

4. Discussion

Overall, data from this study showed that in focal DRE patients a short-term real TNS was able to induce a consistent and significant increase of the absolute power of alpha band, particularly localized in the parietal-occipital areas. This effect reached its largest extent in the first 4 min of TNS, then gradually decreased and disappeared after 15 min of stimulation. Data from a control experiment revealed that the observed effect of stimulation on EEG was specific to TNS stimulation since it was not found during MNS.

A modulation of EEG alpha absolute power was previously described following chronic stimulation of the vagus nerve in epileptic patients [21]. In their work the authors demonstrated that VNS induced a better structured composition of the background EEG, while enhancing the power of EEG rhythms which characterize each sleep-wake state (i.e. delta and theta in non-REM sleep, alpha in both REM sleep and wakefulness). These authors suggested that VNS is able to induce an improvement of cortical electrogenesis, thus enhancing the brain's ability to generate a normal electrical activity, possibly through metabolic changes of the thalamo-cortical EEGl generating system.

Neuroimaging human studies reported that an increased EEG alpha power correlates with a decreased metabolic rate in multiple regions of the occipital, superior temporal, inferior frontal and cingulate cortex, but with an increased activity in the thalamus and insula [34,35].

Several works indicate, as a functional aspect of the alpha rhythm, that an increased amplitude of regional alpha would represent a cortical inhibitory process useful to control cortical activation and excitability in a top-down manner [34,36]. Moreover, the increase in alpha power across sensorimotor regions showed a strong inhibitory influence on the generation of neuronal firing and action potentials in monkey models [37]. Since a high level of excitatory neurotransmission could be a neurobiological factor that may underlie augmented susceptibility to develop pharmaco-resistance [38], a possible increase of inhibitory cortical activity may underpin the clinical antiepileptic effect of TNS.

The TNS-induced increase of the alpha absolute power was proved to be specific, since it was not detected following MNS. This specific effect of TNS could be explained in terms of the connections that the trigeminal afferent fibers establish within the brainstem. Indeed it is well known that like VNS, TNS, ultimately influences the pattern of neuronal activity while connecting to brain areas which are thought to modulate the lateral reticular formation, such as the nucleus tractus solitarii and the locus coeruleus [25]. These are, in turn, connected to noradrenergic and serotoninergic systems associated with the regulation of mood, anxiety [39] and to glutamatergic and GABAergic systems regulating the brain susceptibility to seizures [40]. The nucleus tractus solitarii and the locus coeruleus are also considered as nuclei which disseminate neuromodulatory compound in the central nervous system, since they profoundly affect its excitability at virtually all levels and are believed to play a key role in mediating the clinical benefits observed following TNS [25,41–43].

Importantly, sensory input relayed via the trigeminal nerve to the ventral posterior medial thalamus, could also trigger intra-thalamic inhibition within a given nucleus or activate the inhibitory cells that comprise the reticular nucleus of the thalamus [23]. Therefore, it is possible that afferent trigeminal input arising from TNS could also

Table 2
Mean frequency and absolute power measured for the frequency of each band (delta, theta, alpha, beta and gamma) before (PRE) during and after (POST) short.term transcutaneous trigeminal nerve stimulation (TNS).

Variable	Band	Derivation	Time (PRE, DURING, POST)	Condition (real-TNS, sham-TNS)	Time × Condition	Derivation × Condition	Derivation × Time	Derivation × Time × Condition
Absolute Power	Delta (0.5–3.0 Hz)	$F_{17,340} = 4.440$ $p < 0.001$	$F_{2,40} = 0.837$ $p = 0.44$	$F_{1,20} = 3.197$ $p = 0.09$	$F_{2,40} = 0.362$ $p = 0.70$	$F_{17,340} = 0.756$ $p = 0.74$	$F_{34,680} = 0.772$ $p = 0.84$	$F_{34,680} = 1.181$ $p = 0.22$
	Theta (3.1–7.0 Hz)	$F_{17,340} = 4.785$ $p < 0.001$	$F_{2,40} = 1.269$ $p = 0.29$	$F_{1,20} = 0.250$ $p = 0.62$	$F_{2,40} = 0.187$ $p = 0.83$	$F_{17,340} = 0.819$ $p = 0.671$	$F_{34,680} = 1.052$ $p = 0.39$	$F_{34,680} = 1.339$ $p = 0.09$
	Alpha (7.1–13.0 Hz)	$F_{17,340} = 11.033$ $p < 0.001$	$F_{2,40} = 3.445$ $p = 0.04$	$F_{1,20} = 0.397$ $p = 0.54$	$F_{2,40} = 1.676$ $p = 0.20$	$F_{17,340} = 0.966$ $p = 0.496$	$F_{34,680} = 2.208$ $p = 0.001$	$F_{34,680} = 1.748$ $p = 0.006$
	Beta (13.1–30.0 Hz)	$F_{17,340} = 6.302$ $p < 0.001$	$F_{2,40} = 1.308$ $p = 0.282$	$F_{1,20} = 0.028$ $p = 0.868$	$F_{2,40} = 2.416$ $p = 0.10$	$F_{17,340} = 0.516$ $p = 0.94$	$F_{34,680} = 1.099$ $p = 0.32$	$F_{34,680} = 1.181$ $p = 0.22$
	Gamma (30.1–48.0 Hz)	$F_{17,340} = 9.128$ $p < 0.001$	$F_{2,40} = 2.092$ $p = 0.14$	$F_{1,20} = 0.742$ $p = 0.40$	$F_{2,40} = 1.117$ $p = 0.21$	$F_{17,340} = 0.739$ $p = 0.76$	$F_{34,680} = 1.036$ $p = 0.41$	$F_{34,680} = 1.091$ $p = 0.33$
Mean frequency (0.5–48.0 Hz)		$F_{17,340} = 5.669$ $p < 0.001$	$F_{2,40} = 1.873$ $p = 0.17$	$F_{1,20} = 1.238$ $p = 0.28$	$F_{2,40} = 1.199$ $p = 0.31$	$F_{17,340} = 1.475$ $p = 0.10$	$F_{34,680} = 0.817$ $p = 0.76$	$F_{34,680} = 0.725$ $p = 0.88$

The table reports the results of ANOVA considering the between and within-subject main effect of Derivation, Time, Condition and interactions among factors.

evoke sufficient inhibition to interrupt any pathological oscillatory activity within the thalamus that could contribute to seizure activity [23]. In this view, the alpha-activity oscillation in the awake brain, modulated by thalamo-cortical firing and brainstem afferent inputs [44,45], may act as a seizure-preventing mechanism, inhibiting cortical abnormal excitability and ictal spreading. Indeed, the alpha-power enhancement seems to favor a talamo-cortical stabilization [46] similar to the "transient functional deafferentation" achieved during phases of stable synchronized Non-REM sleep (i.e. 'closed thalamic gate' theory) in which, generally, seizures are known not to occur [47].

Moreover, some recent studies from intracranial EEG recording in epileptic patients demonstrated that the enhancement of alpha activity is able to modulate and temporally break ongoing gamma activity, which has been found to often precede epileptiform discharges [48,49], with a mechanism of regional pulsed inhibition [50,51]. In line with these data, it has been reported that the alpha rhythm enhancement may be considered as a sign of cortical "functional inhibition", generated by modulation of subcortical structures [34,45,52].

In keeping with the hypothesis of Diaz and co-workers [54], a possible explanation of our results is that in patients with focal DRE, some unpredictable changes in the intrinsic dynamic properties of neural activity have led to a global reorganization across frequency power, possibly with a lowering in alpha band, which may reflect a dysfunction in thalamic–cortical circuits and eventually affect the cortical susceptibility to initiate seizures [36,53,54]. Over this substrate TNS could exert its effect by globally enhancing alpha power and so restoring the normal balance between excitatory and inhibitory mechanisms.

No significant effects of TNS were identified either for other frequency bands (delta, theta, beta and gamma) or for qualitative analyses (IEDs detection). This result is in line with previous works showing that the clinical improvement observed following cranial nerve stimulation was detected even without significant correlated EEG interictal changes [21,22,55]. An inconsistent effect on IEDs following acute or chronic VNS was also described [21,55,56]. Moreover, it is well known from clinical evidences that seizure reduction/freedom, and the AEDs clinical response, does not necessary follow or reflect IEDs EEG improvement or normalization [57,58]. The ictal and interictal phenomena should be seen therefore as governed by different cellular mechanisms [59]. Furthermore, it may be possible that short-term TNS is not able to acutely induce any changes on IEDs that could happen, instead, with chronic intervention which may act with an "accumulation effect" and lead to a sustained network remodulation [21,60,61].

The present study is the first documenting of the specific effect of short-term TNS on EEG activity in epileptic patients. However, some limitations have to be acknowledged. A cross-over design was more appropriate to investigate the effect of an intervention in a heterogeneous population, such as DRE patients. This design was not applicable in our study, since 85% of the subjects were not available to come back for a second experimental session. Furthermore, since in our study this TNS effect has been documented only for the acute stimulus protocol in a relative small patient group, it may be worthwhile for further TNS studies focused on the EEG chronic effect in epileptic subjects, so as to provide new insights into the mechanisms underpinning the TNS

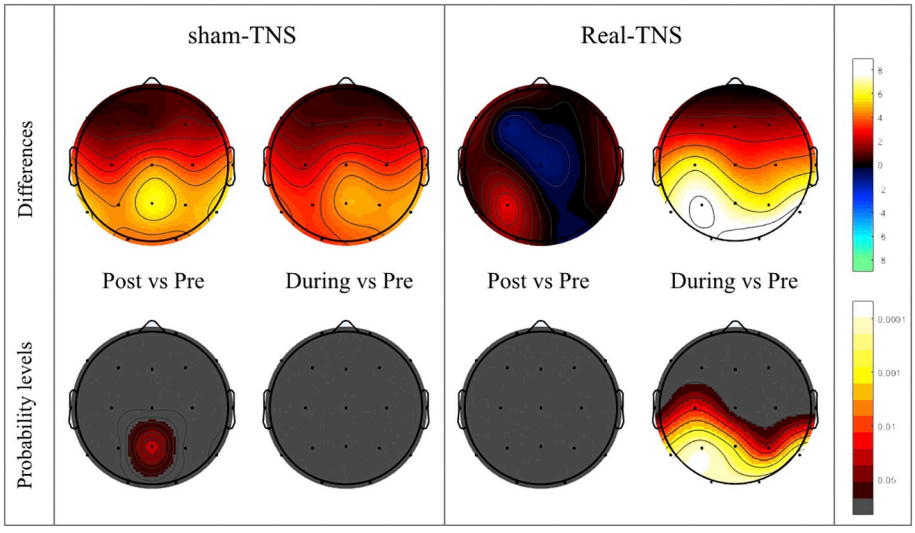

Fig. 1. Variations of absolute alpha power (7.1–13 Hz) associated with different EEG phases and different conditions. Top row: power differences between an experimental condition (post-TNS or During-TNS) and the reference condition (Pre-TNS). Bottom row: the associated probability levels for post-hoc comparison of marginal means (estimated by Tukey's HSD - Honestly Significant Difference - method) following repeated-measurements analysis or variance (associated significant level for group * time * region interaction: $p = 0.006$).

antiepileptic effects.

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Declarations of interest

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Author contributions

ST and FD conceived the study; ST, FG and CF performed the experiments and analyzed the data; FG and FDC performed the statistical analysis; CF and FG drafted the manuscript; FD, GPS, FDC, BM and ST revised critically the manuscript.

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