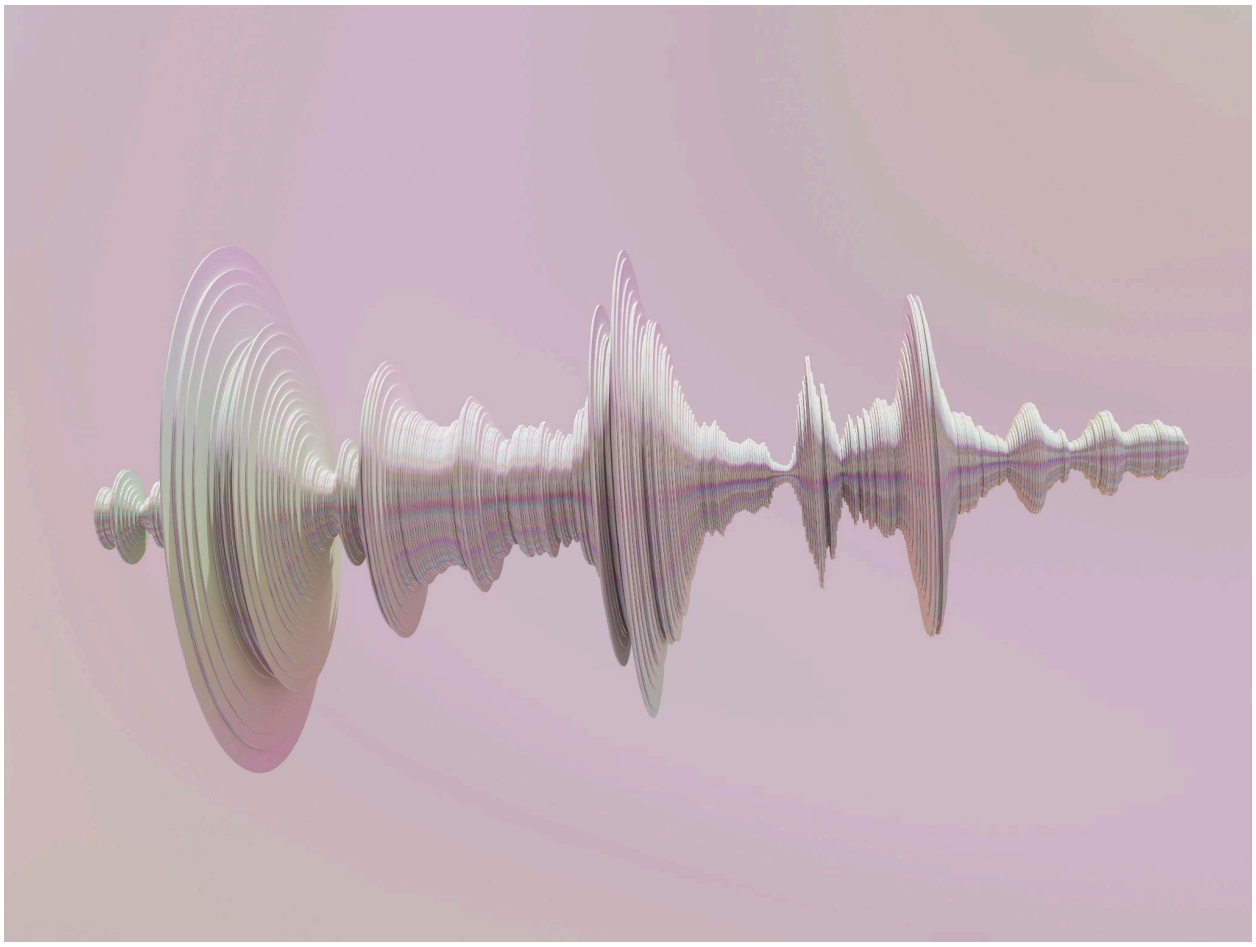
Speech Waves

Determining Functionally-Relevant Neural Oscillations For Speech



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Abstract

This study explored whether transcranial alternating current stimulation (tACS) could modulate vocal vibrato in typical speakers, and to examine which stimulation intensities are effective at eliciting these modulations. Vocal vibrato was chosen as a non-pathological model for vocal tremor in Parkinson's disease (PD), as they share similar acoustic mechanisms. Four typical female speakers produced vibrato vocalizations while undergoing tACS at their individual vibrato frequency, with intensity conditions including 1, 2 and 3 milliampere (mA, peak-to-peak) and a sham. The results revealed that 1 mA tACS led to an increase in vibrato frequency rate, extent, and variability. 2 mA showed more modest increases for vibrato frequency extent and variability, and 3 mA didn't show any consistent changes. These findings support the idea that moderate-intensity tACS can help desynchronize the neural oscillations that play a role in vocal motor control. However, no consistent effects were observed on amplitude vibrato metrics, which might be due to different underlying neural mechanisms. Limitations include the small sample size (n = 4) and the individual variability among participants, which limit generalizability. However, the findings provide encouraging insights into how tACS could potentially aid in treating vocal tremor in PD in the future.

Preface

This thesis was written as part of my minor thesis research project during the first year of the Research Master's programme in Behavioural and Cognitive Neurosciences (BCN), and it is a reflection of my academic interest as well as my personal ambition to explore the intersection between the brain and movement disorders. With an undergraduate degree in Human Movement Sciences, I have always been fascinated by the neural basis of movement and its disruption in conditions such as Parkinson's disease. This project offered the chance to combine that interest with my growing passion for brain stimulation techniques, in this case transcranial alternating current stimulation (tACS), here applied in the domain of speech.

Along the way, I came to really value an aspect of research that I had previously tended to overlook: the amount of time and attention devoted to developing a good research design. Planning, refining, and justifying each element of the study not only taught me patience, but gave me greater respect for the work researchers do behind the scenes, often well before any data is collected.

I also want to express my sincerest gratitude to my supervisors, Dr. Miles Wischnewski and Dr. Defne Abur. Their feedback, support, encouragement, and the many opportunities they offered me, both through thesis and beyond, have been invaluable. With Miles, I was given the opportunity to do a literature review that became my first publication and also learned more about tACS, and with Defne, I attended my first two academic conferences, where I was lucky enough to receive a poster prize for the second time. The first prize was at the BCN Annual Member Day, where all the students presented their upcoming research. These experiences have contributed significantly to my academic as well as personal development.

Finally, I would like to mention that limited use of artificial intelligence (e.g., ChatGPT) was made during the writing of this thesis. It was used solely for language refinement. All content, structure, argumentation, and analyses are entirely my own.

Samira Barzegar
Groningen, June 2025

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Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting over 10 million people worldwide, with projections reaching 25 million by 2050, which poses a significant public health challenge (Su et al., 2025). A hallmark symptom of PD is tremor–rhythmic, involuntary shaking, typically affecting the limbs. Less recognized is vocal tremor, which affects the voice in a similar way (Gillivan-Murphy et al., 2016). In fact, vocal and speech-related impairments, collectively referred to as hypokinetic dysarthria, are among the earliest signs of PD (Pawlukowska et al., 2015), and affect up to 90% of individuals with PD (Ho et al., 1999). Among these impairments, vocal tremor, marked by involuntary, periodic fluctuations in pitch and loudness (Gillivan-Murphy et al., 2018), affects up to 68% of people with PD (Gillivan-Murphy et al., 2018) and can severely impair communication and quality of life (Miller et al., 2006). Although often grouped with limb tremors, vocal tremor may involve distinct mechanisms (Tykalová et al., 2020) and remains poorly understood due to limited focused research and inconsistent evidence regarding its time of onset, anatomical source, and underlying mechanisms (Perez et al., 1996; Holmes et al., 2000; Broadfoot et al., 2019).

Vocal tremors in PD have been proposed to result from oscillatory movements across multiple structures within the vocal tract, rather than being confined to the vocal folds alone (Gillivan-Murphy et al., 2016). Although rigidity in the laryngeal muscles, detectable as abnormal muscle firing at voice rest, is common in PD, electromyographic studies have not observed tremor activity in these muscles (Zarzur et al., 2013). This absence of peripheral tremor-related activity suggests a possible central or neural origin of vocal tremor.

To better understand tremor, a growing body of research has focused on the role of neural oscillations–rhythmic patterns of brain activity that synchronize neural signals across regions to facilitate smooth motor coordination (Foffani & Alegre, 2022). In speech, oscillations help coordinate activity across a distributed network involving motor and language-related areas such as Broca's area, the motor cortex, the supplementary motor area, the basal ganglia, the cerebellum, and the insula (Price, 2012). Importantly, sensory areas, especially the auditory cortex, also contribute to this network by providing real-time feedback that informs and adjusts vocal output, which supports precise speech modulation (Abur et al., 2021). Together, these motor and sensory systems collaborate to plan, initiate, and fine-tune speech, with neural oscillations facilitating their interaction by integrating sensory feedback with motor commands to ensure fluid and accurate vocal production. For instance, the *Directions Into Velocities of Articulators* (DIVA) model proposes that Broca's area maps speech sounds onto motor commands; the motor cortex executes these commands via articulatory muscles; and the cerebellum ensures smooth timing through feedback, particularly from auditory regions (Guenther, 2006). Oscillations in different frequency bands contribute to motor control: beta (13-30 Hz) supports motor planning and execution (Zaepffel et al., 2013), gamma (> 30 Hz) integrates sensory input with motor output (Ulloa, 2022), and theta (4-8 Hz) links sensory processing with movement planning (Karakaş, 2020). These oscillatory patterns are essential for the fine control of voluntary movements, including speech.

Any disruptions in these neural oscillatory patterns can lead to motor impairments, as regions of the brain fail to coordinate their activity in a synchronized manner. In PD, motor impairments arise from abnormal beta oscillations, particularly within the basal ganglia-thalamo-cortical loop (Asadi et al., 2022). This disruption becomes most evident in tremors, where the pathological neural oscillations manifest themselves in the oscillatory nature of the involuntary, rhythmic movements in the limbs or voice (Gillivan-Murphy et al., 2016). This observation demonstrates the critical role of neural oscillations in maintaining smooth and coordinated motor and speech control.

Given the central role of oscillations, neuromodulation techniques targeting neural oscillations are gaining attention as potential treatments. One such approach is transcranial alternating current stimulation (tACS), a non-invasive method that applies weak oscillating currents to the scalp. These currents generate electric fields in the brain that can influence neural spike timing, which leads to changes in endogenous oscillations, known as entrainment (Wischnewski et al., 2022). Entrainment typically occurs at higher induced intensities (> 0.3 mV mm^{-1}), whereas lower induced intensities (< 0.3 mV mm^{-1}) tend to desynchronize neural firing (Wischnewski et al., 2019; Alekseichuk et al., 2022; Krause et al., 2022; Zhao et al., 2024). tACS has shown potential in modulating motor and cognitive functions across disorders including Alzheimer's disease (Benussi et al., 2022), depression (Haller et al., 2020) and importantly, PD (Guerra et al., 2021). One study achieved a 50% reduction in resting hand tremor by applying tACS over the motor cortex, and adjusting the frequency and timing in real-time to the tremor, aiming to desynchronize the neural oscillations driving the tremor (Brittain et al., 2013). Another study applied cerebellar tACS at tremor frequency and significantly reduced hand tremors by up to 76% during stimulation and 68% post-stimulation (Rahimi et al., 2023).

The ability of tACS to modulate abnormal oscillations associated with limb tremors suggests potential for treating vocal tremors, which currently has limited effective therapies. Although levodopa and DBS improve general motor symptoms, they often fail to improve vocal tremor and may even worsen speech (Gibbins et al., 2017; Tsuboi et al., 2014). Other options, including propranolol, primidone (Richards, 2017), and speech therapies like the Lee Silverman Voice Treatment (LSVT; Cnockaert et al., 2007), show only inconsistent or temporary relief. These limitations highlight the need for alternative treatments. tACS presents a promising, non-invasive option, yet research on its use for speech, and vocal speech specifically, remains scarce.

To address the lack of research on the effects of tACS on vocal tremor, we propose using vocal vibrato as a mechanistic, non-pathological model for the investigation of pathological vocal tremor. Vocal vibrato is a natural, rhythmic oscillation in pitch and loudness during sustained vocalization, typically ranging from 4.5 to 8 Hz (Reddy & Subramanian, 2015). It often emerges automatically in singers as voice training and development progresses c. Importantly, Ramig and Shipp (1987) showed that vocal vibrato and vocal tremor in PD share similar acoustic properties, differing only slightly in frequency and amplitude. They hypothesized that vibrato and tremor lie on a continuum, where vibrato represents a normally suppressed motor activity that singers can voluntarily control, but which becomes involuntary and pathological in PD. Because singers can voluntarily produce vibrato, it offers a practical and ethical model for early-stage, exploratory testing of the effects of tACS on

tremor-like vocal oscillations, before translating findings to clinical populations. This approach may ultimately guide the development of interventions aimed at improving speech motor control and communication in PD.

The aim of this study was to determine whether transcranial alternating current stimulation (tACS) can modulate vocal vibrato, and to examine how different tACS intensities influence this modulation. Vocal vibrato is used as a non-pathological model for vocal tremor in Parkinson's disease (PD) in the present study, due to shared acoustic and mechanistic features (Ramig & Shipp, 1987). Typical speakers capable of producing vibrato underwent four stimulation conditions, including three tACS intensities and a sham (control). During each condition, participants vocalized intermittent vibrato while receiving tACS at a frequency matched to their individual vibrato rate. Acoustic data were analyzed to assess changes in vibrato frequency, amplitude and variability. Building on evidence that tACS can reduce hand tremors (Brittain et al., 2013), we hypothesize that it may also modulate vocal vibrato, given its tremor-like characteristics. According to prior studies showing that lower tACS intensities tend to desynchronize neural activity, while higher intensities enhance entrainment (Wischnewski et al., 2019; Alekseichuk et al., 2022; Krause et al., 2022; Zhao et al., 2024), we expect increased vibrato variability at lower intensities and reduced variability at higher intensities, reflecting more synchronized, tremor-like stability. If supported, these findings would show that tACS can modulate vocal control in a controlled manner and ultimately inform therapeutic strategies for vocal tremor in PD.

Methods

Study Design

The experiment followed a single-session, single-blinded, within-subjects design lasting approximately 120 minutes per participant.

Participants

Four typical female participants (ages 20-27 years; M = 23.5, SD = 3.16) were recruited using flyers and word-of-mouth advertising. Participants were selected based on their ability to produce vocal vibrato and were required to meet the following inclusion criteria, as assessed by a brief questionnaire: native speaker of a Germanic language (e.g., Dutch, English, German), no skin conditions (e.g., eczema, psoriasis) or tattoos on the scalp or near the stimulation areas (above the ears/temporal regions), no history of being ill in the past two weeks, and no history of concussion or loss of consciousness from head injury. Participants were excluded if they had a history of epilepsy, migraines, any speech, language, hearing, or neurological disorder, sensitivity to electrical stimulation, or were taking psychoactive medications.

All participants provided written informed consent. The study was approved by the Research Ethics Review Committee (CETO) of the University of Groningen (Reference: 103589787) and was conducted in accordance with the Declaration of Helsinki.

Screenings

Pre-experiment Questionnaire: A brief screening questionnaire in paper format assessed the inclusion/exclusion criteria to ensure eligibility and safety for transcranial alternating current stimulation (tACS). This questionnaire can be found in Appendix A.

Hearing Screening: Participants completed a hearing screening to confirm normal auditory function. They were asked to detect tones at -25 decibels Sound Pressure Level (dB SPL) across six frequencies (125, 250, 500, 1000, 2000, and 4000 hertz (Hz)).

Only participants who passed both screenings continued to the experimental session.

Experimental Setup and Calibration

All recordings took place in a sound-attenuated booth. Participants wore an omnidirectional, over-the-ear microphone (MX153, Shure) over their right ear, positioned 7.5 cm from the mouth at a 45 degree angle from the mouth to ensure consistency across conditions and participants. The microphone was attached to the cheek using medical tape for stability. Signals were acquired via a soundcard (Microbook IIc, MOTU) via a custom MATLAB (v2024b) script.

To ensure sound intensity measurements from the speech signal were accurate, the experimental sound intensity was calibrated using a recording in the sound booth. Namely, the intensity of a 3-second tone (delivered via an electrolarynx; Harmony™, Labex) placed

at the mouth was recorded and saved via MATLAB (v2024b), and simultaneously measured in the booth using a sound level meter (AZ-8922) placed at the microphone. After each participant's data collection, all signal intensities were converted to the "true" intensity in the sound booth using the values from the calibration procedure.

Data Collection Procedures

Electrode Placement and tACS Protocol

tACS was administered using a NeuroConn DC Stimulator Plus device with two 3x3 cm rubber electrodes. Stimulation targeted the left fronto-temporal-parietal cortex, covering speech-related brain areas, including Broca's and Wernicke's areas, as well as primary and secondary auditory areas. Electrode locations were determined using electric field modeling performed in SimNIBS (v3.2), which identified optimal coverage using positions F7-FT7 (frontotemporal) and P3 (parietal), according to the international 10-20 electroencephalography (EEG) system (Figure 1A).

Placement was standardized using anatomical landmarks and measuring tape to ensure consistency across participants.

A custom MATLAB (v2024b) script guided electrode placement by calculating electrode positions relative to Cz using individual head measurements (nasion-inion and inter-tragi distances), which were given as input: F7/FT7 was placed at 40% of inter-tragi distance to the left and 10% of nasion-inion distance forward; P3 was positioned 20% to the left and 20% backward.

Prior to application of the electrodes, the scalp was cleaned with abrasive NuPrep gel, and electrodes were covered evenly with conductive Ten20 paste to ensure secure placement and minimize impedance, which was maintained below 10 kΩ (following prior studies: Liu et al., 2025; Dallmer-Zerbe et al., 2020; Zhou et al., 2024) throughout the experiment. Electrode sites were marked with a washable eye pencil to ensure consistency. Electrodes were disinfected before and after each session using alcohol wipes.

Stimulation conditions were: 1 milliampere (mA), 2 mA, 3 mA (peak-to-peak), and sham. To ensure participant safety and minimize risk, stimulation intensity was gradually increased across conditions. The first condition was always at 1 mA, and the final condition at 3 mA, while the order of the 2 mA and sham condition was randomized. Stimulation was ramped up over 30 seconds at the start of each condition and was administered at each participant's individual baseline vibrato frequency (6.13 ± 0.95 Hz). We used a 60-second ramp-up for one participant, due to slight discomfort during the experiment. In the sham condition, the same electrode placement and ramp-up (to 2 mA) were used, but the current was ramped down immediately after 30 seconds, resulting in no effective stimulation during the condition. During active stimulation tACS was applied for the duration of vibrato production blocks (~8 minutes).

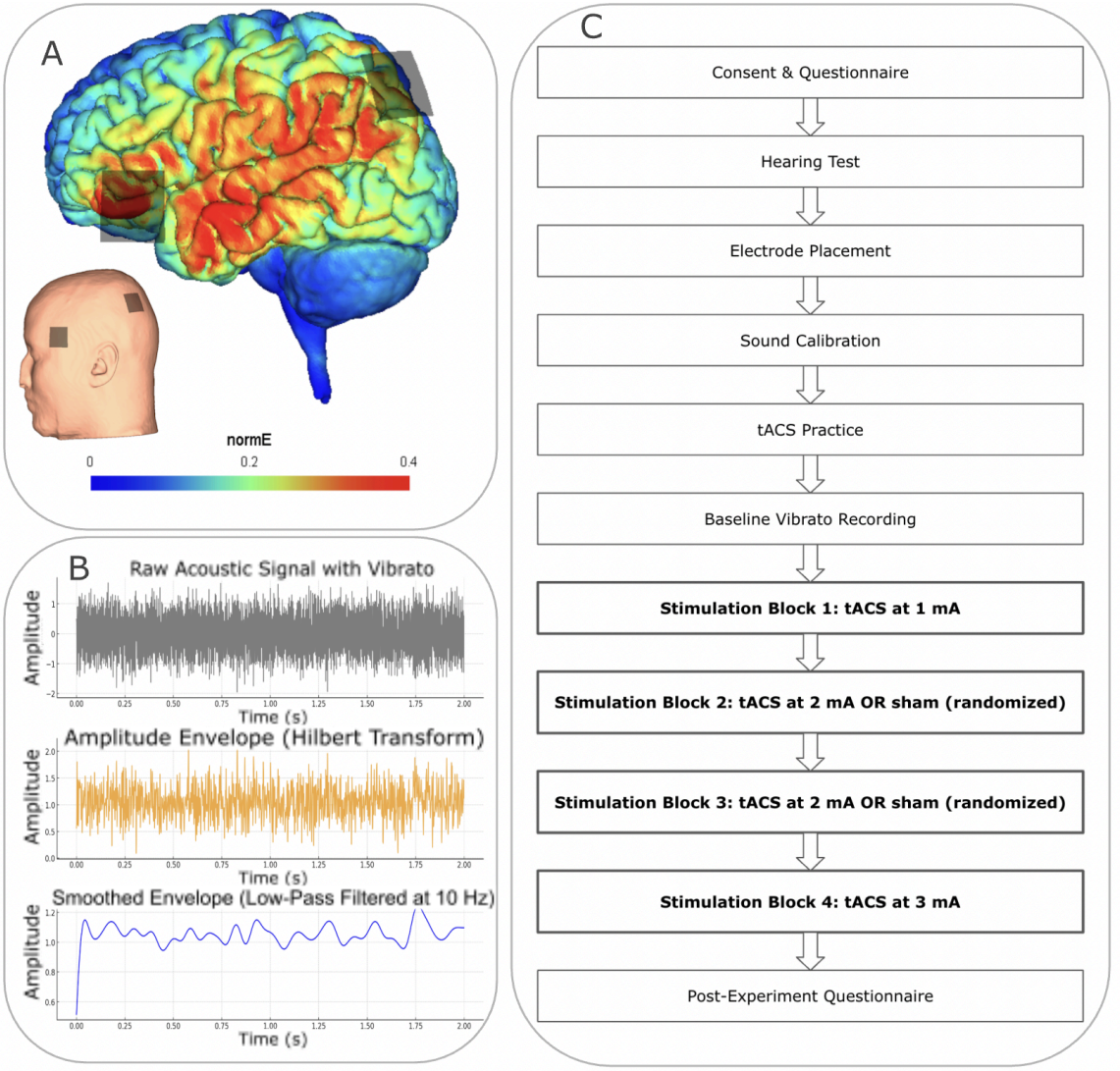

Figure 1 *(A) Simulated electric field heatmap showing the distribution of the induced electric field (expressed in millivolts per millimeter, mV/mm) based on transcranial alternating current stimulation (tACS) at 3 milliampere (mA). Electrodes were placed over standard electroencephalography (EEG) 10-20 system locations F7/FT7 (left frontotemporal) and P3 (left parietal). (B) Acoustic signal processing steps for vocal vibrato extraction. All panels share the same x-axis: time (seconds), and y-axis: amplitude (arbitrary units, normalized digital audio signal). Top panel: raw synthetic vibrato waveform. Middle panel: amplitude envelope extracted via Hilbert transform. Bottom panel: envelope after low-pass filtering at 10 hertz (Hz). (C) Experimental protocol timeline showing the sequence of tasks.*

Baseline Vibrato Measurement

Participants completed five trials sustaining the vowel /a/ with vibrato and five without. The custom MATLAB (v2024b) script automatically calculated vibrato frequency from the five vibrato trials by extracting the amplitude envelope of the audio signal using the Hilbert transform, followed by smoothing with a 10 Hertz (Hz) 4th-order Butterworth low-pass filter to isolate the vocal vibrato (see Figure 1B for illustration of the processing steps). Peaks in

the smoothed envelope were detected to compute the vibrato frequency per trial. The script then averaged these values to determine the participant's overall vibrato frequency, which determined the individualized stimulation frequency, and calculated the standard deviation (SD) across trials to assess consistency.

Main Task Procedure

Participants sat in the sound booth and followed visual cues on a screen to produce vibrato. Each condition consisted of 60 trials: 3 seconds of vocalizing /a/ with vibrato, followed by 5 seconds of rest. A 2-minute rest to drink water was given between conditions. Participants were single-blinded to the stimulation conditions. A 1-minute practice stimulation at 1 mA was given before the main experiment to familiarize the participants with the sensation. Participants were informed about possible side effects, including mild itching/tingling, sensation of warmth, slight tapping under the electrodes and the perception of flickering lights (phosphenes). If any discomfort or impedance issues occurred during the ramp-up, electrodes were pressed firmly to reduce impedance and alleviate any issues.

A custom MATLAB (v2024b) script automated the electrode localization, vibrato frequency calculation, sound calibration (all mentioned above), and the presentation of visual cues on the screen, prompting the participants when to vocalize or rest. All acoustic recordings were automatically saved for later analysis.

Following the experiment, participants completed a post-experiment questionnaire (Appendix B) assessing discomfort, awareness of stimulation, and the ability to identify the sham condition. Blinding effectiveness was evaluated by comparing guesses to actual conditions. A timeline of the entire protocol is shown in Figure 1C.

Data Analysis

Single-trial vibrato recordings were processed using a custom MATLAB (v2024b) pipeline designed to extract six vibrato metrics for both frequency and amplitude domains (Table 1). These metrics were selected based on prior work on vocal tremor characterization (Gillivan-Murphy et al., 2018) and included measures based on the rate, magnitude and variability of the vocal vibrato.

For each participant, vibrato was analyzed separately for each condition. After manually trimming the audio to remove noise before or after each vibrato vocalization, the amplitude envelope was computed using the Hilbert transform and smoothed with a 10 Hz 4th-order Butterworth low-pass filter to get rid of noise. To ensure meaningful comparisons across trials and conditions, amplitude measures were calibrated using a reference signal (recorded at a known SPL during calibration) by computing the root mean square (RMS) of the calibration audio and applying a dB offset to align all trials to an absolute dB SPL scale. Following calibration, amplitude peaks in the envelope were identified to compute the rate of the oscillations in amplitude, the peak-to-peak extent of the envelope, the relative extent as a percentage of the mean amplitude, and the coefficient of variation (CV) in amplitude.

For pitch-based measures, the fundamental frequency contour was estimated using MATLAB's (v2024b) built-in *pitch* function, which utilizes normalized cross-correlation. This contour was then low-pass filtered at 8 Hz to isolate vibrato. Peaks in the filtered pitch contour were used to compute the rate of oscillations in frequency, the peak-to-peak extent in frequency, the relative extent as a percentage of the mean frequency, and the CV in amplitude. Results were saved and visualized to inspect vibrato features across conditions.

Statistical Analysis

All statistical analyses were performed using RStudio (v2024.09.1+394). Outliers were identified and removed per metric using the 1.5 x interquartile range (IQR) rule. To evaluate the effect of tACS intensity as well as trial number on each vibrato metric, linear mixed-effects models (LMMs) were fitted using RStudio's *lme4* package. The models included tACS intensity (categorical: with sham as the reference level; 1 mA, 2 mA, 3 mA), trial number (numeric), and their interaction as fixed effects. Random effects included random intercepts for participants to account for individual variability.

Residual normality and homoscedasticity were assessed using diagnostic plots (histograms, Q-Q plots, residual vs. fitted values). Statistical significance was determined using a threshold of $p < 0.05$.

Table 1 Vibrato metrics used in the analysis. Hz = Hertz; % = percentage; SD = standard deviation; CV = coefficient of variation

Domain	Metrics	Unit	Description	Calculation Method
Frequency	Rate	Hz	Number of oscillations in frequency/pitch per second	*(n* peaks in pitch contour) / (signal duration)
	Relative extent	%	Peak-to-peak variation in frequency relative to mean	(Max. freq. - Min. freq.) / mean freq. x 100%
	CV	%	Variability/stability in frequency over time	(SD freq. / mean freq.) x 100%
Amplitude	Rate	Hz	Number of oscillations in amplitude/intensity per second	*(n* peaks in amplitude envelope) / (signal duration)
	Relative Extent	%	Peak-to-peak variation in amplitude relative to mean	(Max. amp. - Min. amp.) / mean amp. x 100%
	CV	%	Variability/stability in amplitude over time	(SD amp. / mean amp.) x 100%

Results

Participant Characteristics

Four female participants aged 20-27 years old (M = 23.5, SD = 2.87) were included in the study. All completed the full protocol and showed relatively stable vibrato frequency across baseline trials, with a mean standard deviation of 0.7 Hz (SD = 0.2 Hz). While this is slightly higher than the 0.21-0.60 Hz range reported by Pecoraro et al. (2013) for professional singers, this variability is acceptable given our participants were non-professionals. The baseline vibrato frequency, which determined the tACS frequency, averaged 6.13 Hz (SD = 0.95 Hz). Inferential statistics are supplemented with descriptive analyses to capture individual variability.

Linear Mixed-Effects Modeling

Linear mixed-effects models were conducted for each of the six vibrato metrics to assess the effects of tACS intensity and trial number, and their interaction. Assumptions of normality and homoscedasticity were checked via residual histograms, QQ plots, and residuals vs. fitted plots, and showed no major violations.

The main findings are summarized below. Full details of all notable fixed effects are presented in Table 2. The full model results, including non-significant effects, are provided in Appendix C. Figure 2 displays boxplots for each metric across conditions and participants.

Frequency Rate (Hz; Figure 2A): Frequency rate increased at 1 mA ($p < 0.001$) compared to sham, with no significant differences at 2 mA ($p = 0.376$) or 3 mA ($p = 0.507$).

Relative Extent of Frequency Modulation (%; Figure 2B): Frequency extent increased at 1 mA ($p = 0.003$) and 2 mA ($p = 0.002$), with no difference at 3 mA ($p = 0.990$) relative to sham.

Coefficient of Variation in Frequency (%; Figure 2C): Frequency variability increased at 1 mA ($p = 0.009$) and 2 mA ($p = 0.003$) compared to sham, with no significant difference at 3 mA ($p = 0.507$).

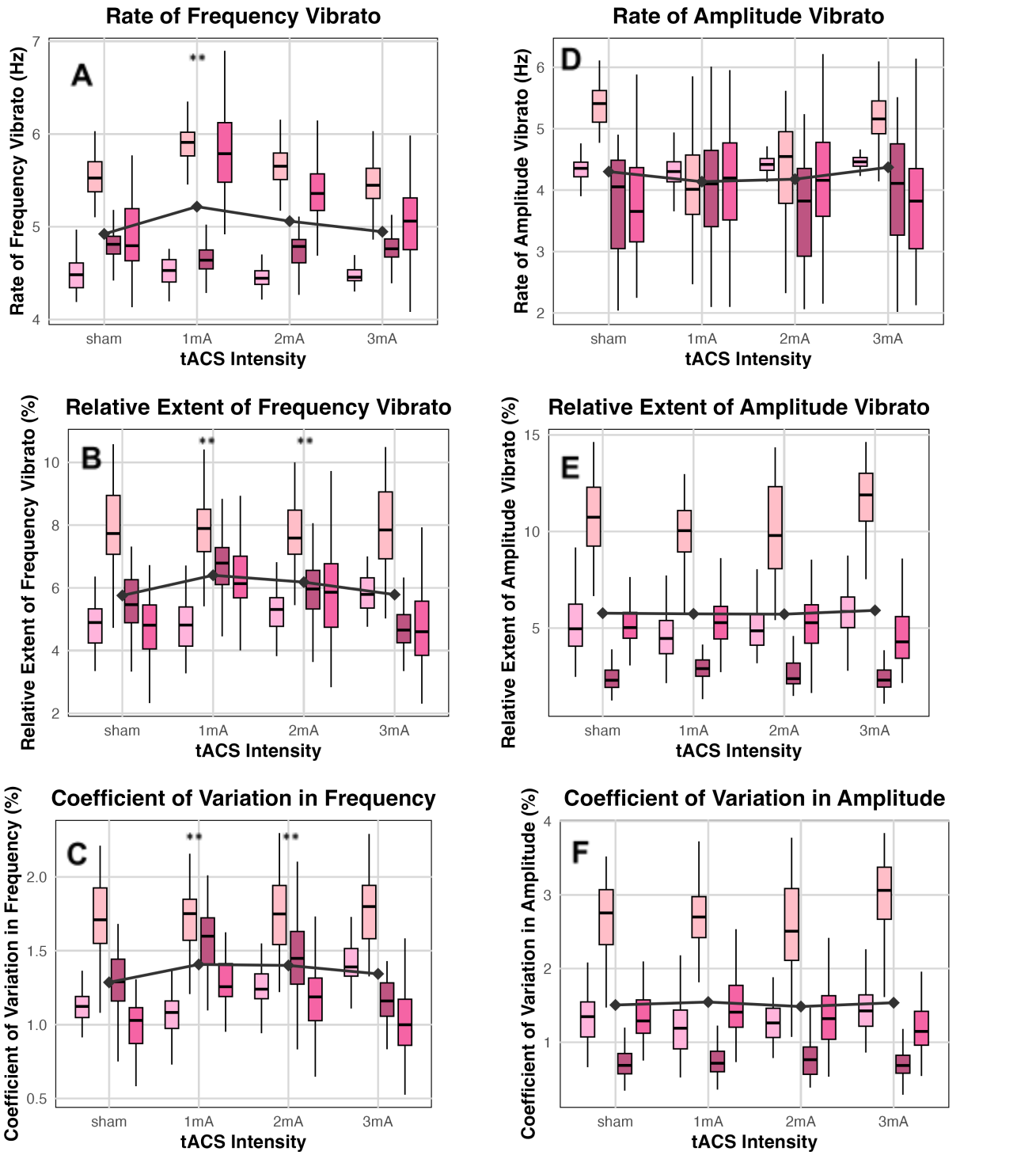

Figure 2 Boxplots of vibrato metrics across tACS intensity conditions for each participant. (A) Frequency Vibrato Rate (Hz) (B) Relative Extent of Frequency Vibrato (%) (C) Coefficient of Variation in Frequency (%) (D) Amplitude Vibrato Rate (Hz) (E) Relative Extent of Amplitude Vibrato (%) (F) Coefficient of Variation in Amplitude (%) Horizontal lines indicate group means; asterisks denote significant differences from sham (** = p < .01).

A negative main effect of trial was observed for frequency rate (p = 0.025), suggesting a slight decrease in frequency rate over trials, regardless of tACS intensity. However, this decrease was fairly small (Table 2). Also, an interaction between trial and tACS intensity was observed for amplitude vibrato rate at 1 mA (p = 0.006; Figure 3).

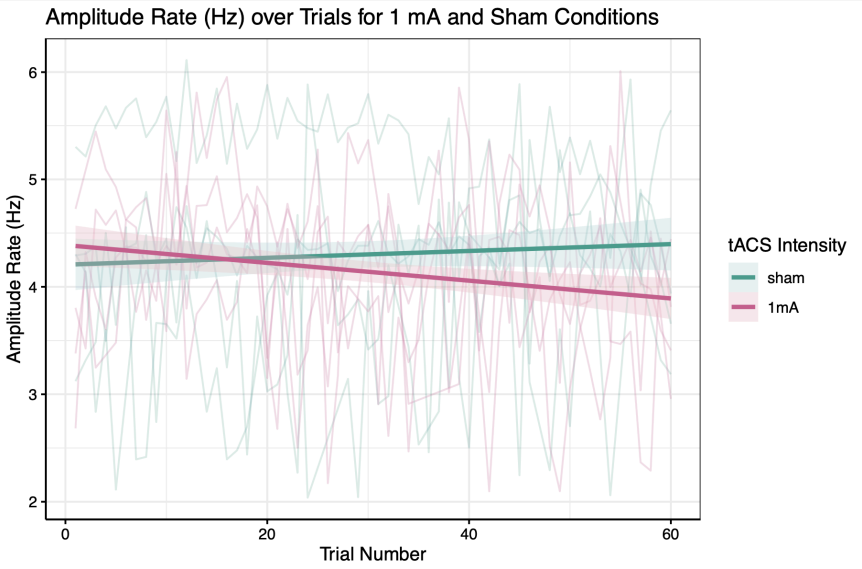

Figure 3 Amplitude Vibrato Rate (Hz) across trials for the 1 mA and sham tACS conditions. Individual participant trajectories are shown with faint lines; bold trend lines represent linear model fits with 95% confidence intervals. The significant interaction was driven by a decreasing slope in the 1 mA condition, suggesting that amplitude vibrato rate tended to decrease over trials, while the sham condition showed a slight increase, suggesting little modulation over time.

Table 2 Key Fixed Effects from Linear Mixed-Effects Models on Vibrato Metrics. *p < .05; **p < .01; ns = not significant

Metric	Term	Estimate (β)	SE	t-value	p-value	Significance
Frequency Rate (Hz)	Intercept (sham)	5.01	0.26	19.1	0.0002	**
	1 mA	0.22	0.06	3.66	0.0003	**
	2 mA	0.05	0.06	0.89	0.376	ns
	3 mA	-0.03	0.06	-0.66	0.507	ns
	Trial	-0.003	0.001	-2.25	0.025	*
Frequency Extent (%)	Intercept (Sham)	5.73	0.63	9.09	0.0017	**
	1 mA	0.70	0.23	3.03	0.0025	**
	2 mA	0.72	0.23	3.11	0.0019	**

	3 mA	0.003	0.23	0.01	0.990	ns
Frequency CV (%)	Intercept (Sham)	1.28	0.14	9.23	0.0018	**
	1 mA	0.11	0.04	2.62	0.0088	**
	2 mA	0.13	0.04	3.01	0.0027	**
	3 mA	0.03	0.04	0.66	0.507	ns
Amplitude Vibrato Rate (Hz)	Intercept (Sham)	4.19	0.22	19.1	0.00001	**
	1 mA	0.19	0.14	1.34	0.181	ns
	2 mA	-0.05	0.14	-0.37	0.708	ns
	3 mA	-0.11	0.14	-0.78	0.436	ns
	Trial	0.003	0.003	1.00	0.317	ns
	1 mA x Trial Interaction	-0.01	0.004	-2.74	0.006	**

Descriptive Patterns Across Participants

Given the small sample size (n = 4), individual data were examined alongside group statistics to capture variability. These individual patterns are visualized in Figure 2, with mean values of vibrato metrics for each participant across conditions presented in Table 3.

Participant 1 showed stable frequency and amplitude vibrato rates (~4.5 Hz), as well as amplitude variability, across intensities. Frequency extent increased moderately at 2 and 3 mA compared to sham, while frequency variability rose only slightly at these intensities. Amplitude extent also showed a subtle increase at 3 mA.

Participant 2 exhibited a slight increase in frequency vibrato rate from sham to 1 mA, while frequency extent and both amplitude and frequency variability remained stable across intensities. Amplitude vibrato rate dipped at 1 mA and then partially recovered at 3 mA, with amplitude extent following a similar dip-and-rise-pattern at 1 mA and 3 mA, respectively.

Participant 3 had stable frequency vibrato rates, but non-linear changes in frequency extent and variability, both peaking at 1 mA before declining. Amplitude vibrato rate and extent fluctuated without observable trends, while amplitude variability remained stable.

Participant 4 showed relatively stable amplitude and frequency vibrato rates, with slight increases at 2 mA and 1 mA, respectively. Frequency extent increased sharply at 1 mA and 2 mA before returning to baseline at 3 mA, while frequency variability only peaked at 1 mA. Amplitude extent and variability fluctuated slightly, both peaking at 1 mA and declining thereafter.

Overall, vibrato rates in both frequency and amplitude domains remained largely stable across intensities. Frequency and amplitude extent showed individual-specific peaks at different intensities. Variability measures fluctuated subtly without consistent trends. These results suggest modest and variable effects of tACS on vibrato metrics, that differ highly across participants and intensities.

Table 3 Mean values of vibrato metrics across tACS intensities for each participant. Hz = Hertz; % = percentage; CV = coefficient of variation

Participant	Condition	Rate of Frequency (Hz)	Frequency Extent (%)	CV of Frequency (%)	Rate of Amplitude (Hz)	Amplitude Extent (%)	CV of Amplitude (%)
1	sham	4.498	4.905	1.129	4.318	5.243	1.360
	1 mA	4.553	4.868	1.078	4.256	4.625	1.208
	2 mA	4.470	5.360	1.255	4.411	5.036	1.287
	3 mA	4.4829	5.891	1.421	4.458	6.001	1.470
2	sham	5.534	7.886	1.734	5.285	11.360	2.761
	1 mA	5.900	7.943	1.707	4.134	9.902	2.722
	2 mA	5.668	7.817	1.758	4.412	10.253	2.669
	3 mA	5.452	8.103	1.775	5.126	12.086	3.193
3	sham	4.792	5.500	1.292	3.952	2.417	0.717
	1 mA	4.630	6.696	1.564	4.110	3.043	0.791
	2 mA	4.721	6.037	1.446	3.856	2.724	0.797
	3 mA	4.763	4.668	1.163	4.203	2.425	0.714
4	sham	4.875	4.859	1.015	3.739	5.216	1.356
	1 mA	5.785	6.409	1.303	4.141	5.452	1.514
	2 mA	5.391	5.862	1.179	4.217	5.288	1.389
	3 mA	5.079	4.765	1.024	3.756	4.574	1.235

Adverse Effects and Blinding Effectiveness

Participants reported sensations during tACS, with tingling being the most common, reported by all participants. Other reported sensations included phosphenes (n = 2), itching (n = 1), left eyelid trembling (n = 1), stinging sensation (n = 1) and vision trembling (n = 1). Discomfort ratings averaged 1.75 (SD = 0.96) on a 5-point scale, indicating minor discomfort. No sensations persisted post-stimulation. All participants reported being able to feel the stimulation during the experiment. When asked whether they could distinguish active stimulation from sham, two participants responded yes with high confidence (ratings of 4 and 5 on a 5-point scale), mentioning burning sensations, tingling, phosphenes, eyelid trembling, and vibrational sensations during active stimulation, which subsided during sham. However, one of them misidentified the 1 mA condition as sham, while the other three participants correctly identified the placebo condition.

Discussion

The aim of this study was to investigate whether transcranial alternating current stimulation (tACS) could modulate vocal vibrato in typical speakers, and to examine which stimulation intensities are effective at eliciting these modulations. Vocal vibrato served as a non-pathological model for vocal tremor in Parkinson's disease (PD) in the present study, as these phenomena are hypothesized to share similar mechanisms (Ramig & Shipp, 1987). To explore whether tACS modulates vocal vibrato, we applied stimulation at individualized vibrato frequencies and analyzed changes in vibrato characteristics across different intensities. Our hypothesis was that lower tACS intensities would increase vibrato variability by inducing neural desynchronization, whereas higher intensities would reduce variability through neural entrainment (Wischnewski et al., 2019; Alekseichuk et al., 2022; Krause et al., 2022; Zhao et al., 2024). Overall, our results provided support for these hypotheses and preliminary insights into the control of vocal vibrato and how tACS might affect it.

At the group level, tACS at 1 mA generally increased the vibrato frequency rate, the extent of frequency modulation, and frequency variability compared to sham, whereas 2 mA only showed small increases in the extent of frequency modulation and frequency variability. However, the higher intensity of 3 mA did not show the expected decrease in variability. Instead, the effects observed at 1 and 2 mA seemed to return back to baseline levels at 3 mA. These findings shed light on how neural entrainment via tACS could potentially affect the motor control of vocal pitch. This topic is still underexplored, with only one study to date using tACS to examine vocal pitch regulation via stimulation of the left inferior frontal gyrus (Li et al., 2022).

The increase in vibrato frequency measures at 1 and 2 mA suggests that this intensity of stimulation may influence the neural processes that control rhythmic pitch modulation. This finding is consistent with previous studies indicating that tACS can alter rhythmic motor behaviors, like hand tremors (Brittain et al., 2013) and finger tapping (Guerra et al., 2018), by entraining the underlying neural oscillations in a frequency-specific manner (Herrmann et al., 2013). The increase in frequency variability at 1 and 2 mA supports the notion that lower to moderate tACS intensities can introduce neural desynchronization, which in turn may heighten oscillatory instability (Wischnewski et al., 2019; Alekseichuk et al., 2022; Krause et al., 2022; Zhao et al., 2024). This destabilization of vibrato variability at moderate tACS intensities may have potential implications for PD, where the degree of excessive synchronization relates to symptom severity (Witcher et al., 2014). By desynchronizing these excessively synchronized oscillations, tACS might reduce the severity of (vocal) tremor. The observed "window" between 1 mA and 2 mA suggests a potential therapeutic range where tACS might destabilize pathological tremor oscillations, although more research is needed to confirm this connection.

The increase in variability of frequency vibrato at 1 mA, less of an increase at 2 mA, and no consistent effects at 3 mA aligns with the expected non-linear dose-response curve, where lower intensities increase variability, intermediate intensities mark a transition phase, and higher intensities increase variability. This aligns with earlier suggestions that the effects of tACS can vary in non-linear ways depending on the intensity (Krause et al., 2022; Zhao et al., 2024). While these findings are still speculative, they underscore the importance of

further research into intensities above 3 mA, as long as safety and tolerability are taken into account, to see if variability starts to decline beyond what seems to be a transitional phase.

While the present study found effects of tACS on several vibrato metrics in the frequency domain, no consistent effects of tACS were found on amplitude vibrato modulation. One possible explanation for this is that amplitude modulation relies on different neural pathways compared to frequency modulation. Previous research indicates that frequency modulation is mainly controlled by the neural activity of the cricothyroid muscle and associated laryngeal adjustments, while amplitude modulation might engage other or additional physiological routes, such as vocal fold tension, respiratory control, and resonance effects (Dromey et al., 2007). Since the left inferior frontal gyrus was one of the targeted regions in this study (Figure 1A), and this region is involved in vocal pitch regulation (Li et al., 2022), our montage may have primarily influenced circuits related to frequency modulation, without adequately targeting those responsible for amplitude modulation. However, this remains speculative.

An additional intriguing observation was the small drop in vibrato frequency rate across trials, regardless of the stimulation intensity. Although this effect was small, it might indicate some vocal fatigue from the repeated production of vibrato, as muscle fatigue can hinder our ability to keep stable tension in the vocal folds (Titze, 1983), possibly leading to changes in vibrato frequency or stability. This highlights the importance of considering time-related effects in vocal control research by incorporating rest breaks and statistically accounting for the number of trials or time as a covariate, as was done in this study.

The present study revealed inter-individual variability in how vibrato rate, extent, and variability changed across stimulation intensities. Such variability is consistent with prior neuromodulation research demonstrating that tACS efficacy depends on individual neuroanatomical and neurophysiological factors (Zanto et al., 2021), including skull thickness, cerebrospinal fluid volume, cortical folding, and local tissue properties (Shahid et al., 2012; Truong et al., 2013; Opitz et al., 2015), which can cause up to a threefold variation in cortical electric field strength from the same stimulation intensity (Datta et al., 2012; Russell et al., 2013). Furthermore, vocal vibrato control involves interactions across cortical, subcortical, and brainstem motor networks (Jürgens, 2002), and individual differences in these systems, as well as variations in vocal training (Mürbe et al., 2006), may further influence individual responsiveness to tACS. These findings highlight the need for personalized stimulation protocols, based on electric field modeling (Kasten et al., 2019), and adaptive tACS setups that adjust parameters in real-time based on feedback, like the one used by Brittain et al. (2013), to account for individual differences and enhance outcomes.

While this study focused on typical speakers, the findings may inform future research on using tACS to address vocal symptoms in PD. Such symptoms can significantly hinder communication by reducing speech intelligibility (Miller, Allcock, et al., 2007), which, in turn, can negatively affect self-esteem and overall quality of life (Miller, Noble, et al., 2007). Building on the similarities between vocal vibrato and tremor (Ramig & Shipp, 1987), our results suggest that tACS had an effect on tremor-like vocal oscillations in typical voices. Although caution is needed when generalizing to PD populations due to the small sample

size and high individual variability, prior success of tACS in managing motor symptoms and tremors in PD (Brittain et al., 2013; Del Felice et al., 2019; Rahimi et al., 2013) support its potential for improving vocal control by desynchronizing the pathological rhythmic neural activity that underlies vocal tremors. Specifically, our finding that moderate tACS intensities increase variability in vocal oscillations suggests a potential mechanism for reducing the stability of tremor-related neural oscillations, thereby alleviating vocal tremor symptoms. These findings suggest a promising direction for future speech therapy and neuromodulation research, with potential applications in PD and other speech-related neurological disorders.

This study has a few limitations. The small sample size (n = 4), limits statistical power. Therefore, results should be interpreted with caution and seen as exploratory. Another limitation concerns the condition order: only the order of the 2 mA and sham conditions were randomized, while 1 mA was always first and 3 mA last. While this semi-fixed sequence was chosen for participant safety and comfort, and to allow for gradual adjustment to the increasing stimulation intensity, it may have introduced possible order effects. While acoustic vibrato analysis was appropriate, future work could include concurrent neurophysiological measures like electroencephalography (EEG; Fehér & Morishima, 2016) to better link cortical entrainment to vocal output. Finally, we did not assess how long stimulation effects lasted, which is an important consideration for clinical application. Currently, the evidence regarding the duration of tACS after-effects is inconclusive, with studies showing effects lasting up to 30 minutes (Neuling et al., 2013), 60 minutes (Kasten et al., 2016; Wischnewski et al., 2018) or even 24 hours post-stimulation (Clancy et al., 2017).

Future research should further investigate the patterns observed in this study, ideally with larger and more diverse samples. If more evidence is gathered, future studies might explore its potential in individuals with PD. To enhance individualized efficacy, adaptive stimulation protocols that adjust intensity or frequency in real-time, based on neural or acoustic feedback, should also be considered (Brittain et al., 2013). Additionally, to better manage any sequence-related issues, future research should consider a fully randomized design. Testing stimulation intensities above 3 mA, with appropriate safety measures to manage discomfort, could clarify whether higher intensities produce the expected reduction in vibrato variability. Pairing tACS with neurophysiological techniques could provide us with a deeper understanding of how stimulation influences vocal motor networks. Lastly, evaluating the long-term effects of (repeated sessions) of stimulation will be essential for moving toward clinical applications in PD.

In conclusion, this study marks an important step towards developing non-invasive neuromodulation therapies for vocal tremor and other speech impairments, particularly in individuals with PD. By showing that moderate-intensity tACS modulated vocal vibrato, this study paves the way for new, targeted interventions aimed at enhancing speech quality and communication for those in clinical settings. The use of an individualized-frequency protocol highlights the relevance of tailoring stimulation protocols to achieve the best therapeutic results. Ultimately, these findings inspire clinical investigations into the potential of tACS to modulate pathological neural oscillations, and they offer promising avenues for the development of innovative and accessible treatments for speech impairments in neurological disorders.

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Appendices

Appendix A: Pre-Experiment Questionnaire

ID#: _____ Date: ____/____/____

1. **Did you sign the consent form?** YES / NO □ if NO, STOP to review and sign the consent form to proceed
2. **What is your sex?** Female /Male / Other:_____
3. **What is your date of birth? ____/____/____**
4. **Are you 16 years old or older?** YES / NO
5. **Do you have any skin conditions or diseases (e.g., eczema, psoriasis) on the scalp?** YES / NO
   If YES, please specify:_____
6. **Do you have any tattoos on the head or near the stimulation areas (above the ears/temporal regions)?** YES / NO
7. **Have you been ill (e.g., fever, flu, infection) in the last 2 weeks?** YES / NO
8. **Have you had a concussion/been unconscious due to head injury in the last 6 months?** YES / NO
   If YES, please describe:_____
9. **Is Dutch your Native language?** YES / NO
   If NO, what is your Native language? _____
10. **Have you ever had a speech, language, or hearing disorder (e.g., stuttering, dysphonia)** YES/NO
    If YES, which ones? _____
11. **Have you ever seen a speech language pathologist?** YES/NO
    If YES, then what was the reason? _____
12. **Do you have a history of neurological or movement disorders (e.g., Parkinson's Disease, essential tremor)?** YES / NO
    If YES, please specify:_____
13. **Have you ever been a singer?** YES / NO
    If YES:
    For how long (years)? _____
    How did you learn? LESSONS / CHOIR/ SELF-TAUGHT
    Do you still sing? YES (CHOIR)/ YES (BAND) / YES (ALONE) / NO
14. **Can you produce a consistent vocal vibrato?** YES / NO / UNSURE
15. **How would you rate your ability to control vibrato? Circle: 1 = very poor, 5 = excellent**
    1 2 3 4 5
16. **Do you have any history of voice training or therapy?** YES / NO
    If YES, please describe:_____
17. **Have you ever participated in brain stimulation studies (e.g., tACS, tDCS, TMS)?** YES / NO
    If YES, which type(s)?_____
18. **Do you have a history of epilepsy, migraines, or sensitivity to electrical stimulation?** YES / NO
    If YES, please specify:_____
19. **Are you currently taking any medications that affect brain activity (e.g., dopamine agonists, antidepressants)?** YES / NO
    If YES, please list:_____

Appendix B: Post-Experiment tACS Questionnaire

ID#: _____ Date: ____/____/____

1. **Did you experience any of the following sensations during the tACS stimulation?** (Please select all that apply)
   - ☐ Tingling
   - ☐ Itching
   - ☐ Redness or irritation on the skin
   - ☐ Headache
   - ☐ Seeing flashes of light (phosphenes)
   - ☐ Dizziness
   - ☐ None of the above
   - ☐ Other (please specify):_____

2. **If you experienced any discomfort, how intense was it?** (1 = very mild, 5 = very strong)

   1 2 3 4 5

3. **Did any of the sensations listed above persist after the stimulation ended?** YES / NO
   If YES, how long did they last?_____

4. **Did you feel the tACS stimulation during the experiment?** YES / NO

5. **Were you able to distinguish between the active tACS and sham (no stimulation) conditions?**

   YES / NO

   If YES, how confident are you in your ability to distinguish between them? (1 = not confident at all, 5 = completely certain)

   1 2 3 4 5

6. **If you were able to distinguish between active and sham tACS, what difference did you notice?** (Optional):_____

7. **Was there anything else about the stimulation or your experience that you'd like to share?** (Optional):_____
   _____

Appendix C: Full Model Results for All Vibrato Metrics

Table C1: Mixed Model Results: Rate of Frequency Vibrato (Hz)

Predictor	Estimate	Std. Error	t-value	p-value
Intercept	5.006	0.262	19.112	0.000
tACS 1 mA	0.218	0.060	3.662	0.000
tACS 2 mA	0.052	0.059	0.886	0.376
tACS 3 mA	-0.039	0.059	-0.663	0.507
Trial	-0.003	0.001	-2.246	0.025
1mA × Trial	0.002	0.002	1.455	0.146
2mA × Trial	0.003	0.002	1.654	0.099
3mA × Trial	0.002	0.002	1.134	0.257

Table C2: Mixed Model Results: Relative Extent of Frequency Vibrato (%)

Predictor	Estimate	Std. Error	t-value	p-value
Intercept	5.731	0.630	9.095	0.002
tACS 1 mA	0.697	0.230	3.031	0.003
tACS 2 mA	0.718	0.230	3.115	0.002
tACS 3 mA	0.003	0.228	0.013	0.990
Trial	0.001	0.005	0.298	0.765
1mA × Trial	0.000	0.007	0.044	0.965
2mA × Trial	-0.007	0.007	-1.117	0.264
3mA × Trial	0.001	0.007	0.227	0.820

Table C3: Mixed Model Results: Coefficient of Variation in Frequency (%)

Predictor	Estimate	Std. Error	t-value	p-value
Intercept	1.276	0.138	9.235	0.002
tACS 1 mA	0.114	0.043	2.624	0.009
tACS 2 mA	0.131	0.044	3.007	0.003
tACS 3 mA	0.029	0.043	0.663	0.507
Trial	0.000	0.001	0.507	0.613
1mA × Trial	0.000	0.001	0.291	0.771
2mA × Trial	0.000	0.001	-0.261	0.794
3mA × Trial	0.001	0.001	0.711	0.477

Table C4: Mixed Model Results: Rate of Amplitude Vibrato (Hz)

Predictor	Estimate	Std. Error	t-value	p-value
Intercept	4.193	0.219	19.146	0.000
tACS 1 mA	0.192	0.144	1.339	0.181
tACS 2 mA	-0.054	0.144	-0.374	0.708
tACS 3 mA	-0.112	0.144	-0.779	0.436
Trial	0.003	0.003	1.002	0.317
1mA × Trial	-0.011	0.004	-2.742	0.006
2mA × Trial	-0.002	0.004	-0.506	0.613
3mA × Trial	0.006	0.004	1.447	0.148

Table C5: Mixed Model Results: Relative Extent of Amplitude Vibrato (%)

Predictor	Estimate	Std. Error	t-value	p-value
Intercept	5.940	1.672	3.553	0.036
tACS 1 mA	0.141	0.289	0.486	0.627
tACS 2 mA	0.027	0.290	0.093	0.926
tACS 3 mA	-0.254	0.292	-0.871	0.384
Trial	-0.001	0.006	-0.161	0.872
1mA × Trial	-0.010	0.008	-1.167	0.243
2mA × Trial	-0.005	0.008	-0.628	0.530
3mA × Trial	0.014	0.008	1.722	0.085

Table C6: Mixed Model Results: Coefficient of Variation in Amplitude (%)

Predictor	Estimate	Std. Error	t-value	p-value
Intercept	1.520	0.420	3.616	0.035
tACS 1 mA	0.132	0.076	1.733	0.084
tACS 2 mA	0.014	0.076	0.186	0.853
tACS 3 mA	-0.059	0.077	-0.765	0.444
Trial	0.000	0.002	0.231	0.817
1mA × Trial	-0.004	0.002	-1.684	0.092
2mA × Trial	-0.001	0.002	-0.528	0.597
3mA × Trial	0.004	0.002	1.786	0.075