Effect of Transcranial Direct Current Stimulation on the Voice

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Study protocol

The present study was approved by the Research Ethics Committee of the institution where the research was conducted (n. 5.758.856). All included volunteers signed the written informed consent prior to the experiment.

Vocally healthy individuals were recruited from the university community. We included volunteers of both sexes, aged 18 to 45 years, who scored up to 16 points on the Vocal Symptoms Scale (VSS), Brazilian version. The Brazilian Dysphonia Screening Tool (Br-DST) was also applied, and those who answered "yes" to both questions would be excluded.

Through a crossover study design, the volunteers underwent four experimental sessions separated by 7 days-minimum washout period to prevent cumulative effects of stimulations. In each session, a single target area was stimulated: (i) left laryngeal M1, (ii) left S1 of the larynx, (iii) left DLPFC, and (iv) cerebellum. The order of sessions was randomized in order counterbalanced by an external researcher to ensure blinding of both volunteer and therapist during vocal assessment. The allocation of sessions was securely concealed within opaque, sealed envelopes that were accessible only to the researcher responsible for tDCS.

The electrode setup during the tDCS sessions was as follows: (i) For left laryngeal M1 , the anode was positioned over C5 (international 10-20 EEG system) and the cathode over the contralateral supraorbital region; (ii) For the left S1 of the larynx, the anode was positioned 3 cm posterior to C5 and the cathode over the contralateral supraorbital region; (iii) For left DLPFC, the anode was positioned over F3 and the cathode over the contralateral supraorbital region; (iv) For the cerebellum, the anode was placed over inion and the cathode over the right deltoid muscle. During the tDCS session, volunteers were instructed to sit in a chair and find a comfortable position. tDCS was delivered by an electrical stimulator (Neuroconn, Germany) through a pair of saline-soaked sponge electrodes (35 cm^2) with a current intensity of 2mA for 20 minutes.

Before and after each session, volunteers were systematically evaluated for vocal acoustic parameters and laryngeal diadochokinesis. The vocal assessment analysis was performed immediately before and after tDCS using the Voxmetria and Vocalgrama software from CTS Informatics. Voice recordings were conducted using an HP Notebook Acer Aspire 5 PC with a Karsect HT-2º headset microphone and Andrea PureAudio™ USB-AS sound signal amplifier equipment. The microphone was positioned four centimeters away from the mouth at a

45-degree angle, with the participant seated and hands resting on their legs, forming a 90-degree angle.

The following tasks were requested: (i) Evaluation of vocal quality through sustained vowel /Ɛ/ for 5 seconds at weak intensity (below 70dB), habitual intensity (between 70 and 80dB), and strong intensity (above 80dB), controlled with a decibel meter. (ii) Cepstral analysis with sustained vowel /Ɛ/ for 5 seconds and the phrase "olha lá o avião azul" at weak, habitual, and strong intensities (as defined above), controlled with a decibel meter. (iii) Diadochokinesis assessment with the task of emitting vowels /a/ and /i/ separately, with rapid and interrupted repetitions of each vowel as fast as possible for eight seconds. (iv) Pitch range assessment through ascending and descending glissandos up to the maximum vocal extension in weak and strong emissions. (v) Assessment of reported vocal effort with sustained vocal effort emission. For phase 1, participants were asked to reproduce a note using a virtual keyboard (3 tones below their maximum glissando frequency) and then maintain that tone while counting from 21 to 30, Immediately afterwards, the participants answered the Adapted Borg CR10 for Vocal Effort Ratings (BR)[17]. Like the original protocol, the Adapted Borg CR10 for Vocal Effort Ratings (BR) maintained a scale from 0 to 10, where 0 means “no vocal effort at all”, and 10 “maximum vocal effort”.

For Vocal range profile, the participants were instructed to produce ascending (from low to high) and descending (from high to low) glissandos of the vowel /ɛ/ as weak as possible and as strong as possible intensities. As the participant produced the vowel /ɛ/ in ascending and descending glissando, the graph was marked with dots that corresponded to the frequency (abscissa) and intensity (ordinate) of the vocal productions. The participants were asked to emit the loudest possible tone during the vocal productions of glissando. The assessment of diadochokinesis was performed by counting peaks per second in the spectrogram, excluding the initial and final two seconds for analysis. For the analysis of acoustic parameters, the recording of the vowel /ɛ/ was edited by discarding the first and last minutes of emission (as these segments showed greater instability), considering approximately three seconds of emission for analysis. The assessment of diadochokinesis was performed by counting peaks per second in the spectrogram, excluding the initial and final two seconds for analysis.

Statistical Analysis

The sample size was determined using the Sample Size Calculators tool designed by David Schoenfeld with the support of the Massachusetts General Hospital - Mallinckrodt General Clinical Research Center (http://hedwig.mgh.harvard.edu/sample_size/js/js_crossover_quant.html).

The calculation was carried out with 12 volunteers, considering the minimum detectable difference (MDD: $1.96 StandardError \sqrt{2}$) and the standard deviation of the primary outcome measures. A statistical power (β) of 80% and a significance level (α) of 5% were considered.

For data analysis, normality was tested using the Shapiro-Wilk test. To compare pre and post stimulation results, the Student's t-test was applied for parametric data and the Wilcoxon signed-rank test for non-parametric data. To compare the effects of tDCS across different time points, a Friedman ANOVA for repeated measures was conducted, followed by pairwise post hoc comparisons with a significance level of 5% ($p < 0.05$). All analyses were performed in SPSS software (version 26.0, SPSS Inc, Chicago, USA).