Dose-dependent response of prefrontal transcranial direct current stimulation on heart rate variability: an electric field modeling study

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Abstract

Transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) modulates the autonomic nervous system by activating deeper brain areas via top-down pathway. However, effects on the nervous system are heterogeneous and may depend on the amount of current that penetrates the brain due to individual brain anatomical differences. Therefore, investigated the variable effects of tDCS on heart rate variability (HRV), a measure of the functional state of the autonomic nervous system. Using three prefrontal tDCS protocols (1.5mA, 3mA and sham), we associated the simulated individual electric field (E-field) magnitude in brain regions of interest with the HRV effects. This was a randomized, double-blinded, sham-controlled and within-subject trial, in which participants received tDCS sessions separated by two weeks. The brain regions of interest were the dorsolateral PFC (DLPFC), anterior cingulate cortex, insula and amygdala. Overall, 37 participants (mean age = 24.3 years, standard deviation = 4.8) were investigated, corresponding to a total of 111 tDCS sessions. The findings suggested that HRV, measured by Root Mean Squared of Successive Differences (RMSSD) and high-frequency HRV (HF-HRV), were significantly increased by the 3.0mA tDCS when compared to sham and 1.5mA. No difference was found between sham and 1.5mA. E-field analysis showed that all brain regions of interest were associated with the HRV outcomes. However, this significance was associated with the protocol intensity, rather than inter-individual anatomical variability. To conclude, our results suggest a dose-dependent effect of tDCS for HRV. Therefore, further research is warranted to investigate the optimal current dose to HRV.

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Abstract

Transcranial direct current stimulation (tDCS) of the prefrontal cortex (PFC) modulates the autonomic nervous system by activating deeper brain areas via top-down pathway. However, effects on the nervous system are heterogeneous and may depend on the amount of current that penetrates the brain due to individual brain anatomical differences. Therefore, we aimed to investigate the variable effects of tDCS on heart rate variability (HRV), a measure of the functional state of the autonomic nervous system. Using three prefrontal tDCS protocols (1.5mA, 3mA and sham), we associated the simulated individual electric field (E-field) magnitude in brain regions of interest with the HRV effects. This was a randomized, double-blinded, sham-controlled and within-subject trial, in which participants received tDCS sessions separated by two weeks. The brain regions of interest were the dorsolateral PFC (DLPFC), anterior cingulate cortex, insula and amygdala. Overall, 37 participants (mean age = 24.3 years, standard deviation = 4.8) were investigated, corresponding to a total of 111 tDCS sessions. The findings suggested that HRV, measured by Root Mean Squared of Successive Differences (RMSSD) and high-frequency HRV (HF-HRV), were significantly increased by the 3.0mA tDCS when compared to sham and 1.5mA. No difference was found between sham and 1.5mA. E-field analysis showed that all brain regions of interest were associated with the HRV outcomes. However, this significance was associated with the protocol intensity, rather than inter-individual anatomical variability. To conclude, our results suggest a dose-dependent effect of tDCS for HRV. Therefore, further research is warranted to investigate the optimal current dose to modulate HRV.

Keywords: transcranial direct current stimulation; heart rate variability; autonomic nervous system; electric field; dose-dependency

Background

Dysregulation in the autonomic nervous system is common in a variety of psychiatric disorders. Heart rate variability (HRV), an index of beat-to-beat variation in the heart, is frequently used to evaluate autonomic (dys)function, and, as such, changes in this measurement are confirmed to be associated with psychiatric illnesses, including depression (Koch et al., 2019)(Borrione et al., 2018).

According to the central autonomic network model, the prefrontal cortex (PFC) and deeper brain regions are involved in high-order autonomic control (Benarroch, 1993). Neuromodulation of the PFC can activate the parasympathetic branch of the autonomic nervous system via top-down influences. This subsequently alters cardiovascular autonomic responses, including HRV (Shaffer et al., 2014)(Thomas et al., 2019). In this sense, previous studies showed that the manipulation of PFC activity using non-invasive brain stimulation (NIBS) techniques can modulate HRV, confirming the prediction of the brain-body connection of central autonomic network theory in humans(Vanderhasselt & Ottaviani, 2022)(Nikolin et al., 2017; Schmaußer et al., 2022).

A NIBS intervention that is particularly promising, due to its safety profile and accessible use, is transcranial direct current stimulation (tDCS). TDCS is able to modulate brain activity via a low-intensity direct electric current applied to the scalp (Lefaucheur & Wendling, 2019). The electric current can increase or decrease cortical excitability in both locally stimulated regions and downstream connected brain networks (Makovac et al., 2017). Although the technique might be able to modulate HRV of healthy and depressive patients by means of targeting the PFC network, the overall findings are still heterogeneous (Razza, Wischnewski, et al.,

2023; Wischnewski et al., 2021). This heterogeneity can be explained in part by interindividual differences in brain morphology, which might alter electric current distributions in the brain (Polanía et al., 2018). Hence, it is plausible that variance in the electric current that penetrates specific brain areas due to individual anatomical variability may underlie the subsequent variance observed in the measured HRV response. Recently, technological developments allow evaluating simulations of the electrical current injected into the brain (Saturnino et al., 2019). While some studies have shown that the simulated electric field (E-field) strength might be associated with cognitive and affective prefrontal tDCS effects(Caulfield et al., 2022; Suen et al., 2020), no study so far has investigated its impact on HRV.

In this study the modulatory effects of distinct prefrontal tDCS intensities (1.5mA, 3mA and sham) on the parasympathetic effects (HRV) is investigated in healthy individuals, and it is explored whether the magnitude of the E-field in brain regions of interest is associated with this outcome. Based on the central autonomic network model, the brain regions of interest were the anterior cingulate cortex (ACC), insula, amygdala and the PFC - focusing on the dorsolateral region. We hypothesize that 1) greater current intensities (1.5mA vs 3.0mA) will increase HRV at a population level (reflecting increased parasympathetic control); 2) individual E-field magnitudes in brain regions of interest will explain inter-individual heterogeneity in HRV modulation, with individuals experiencing relatively higher E-fields demonstrating greater HRV modulation (Wei et al., 2018).

Design

A randomized, double-blind, sham-controlled within-subjects trial design was employed, in which participants received different tDCS protocols (sham tDCS, 1.5mA tDCS, and 3.0mA tDCS). A two-week interval was incorporated between the experimental sessions to mitigate any carryover effects. The research is in line with the Declaration of Helsinki, and it received the approval of the Ethics Committee of the Ghent University Hospital (UZ Ghent - B6702021000839). The study was conducted at the Ghent University Hospital of Ghent University, from March 2022 to March 2023. The current study is part of a larger project (Razza, De Smet, et al., 2023).

Participants

Participants were between 18 to 45 years old without any current or past mental or neuropsychiatric disorder. A psychologist screened all potential participants using the Diagnostic and Statistical Manual of Mental Disorders - version 5 (DSM-5) and the Beck Depression Inventory (BDI) (Beck et al., 1961). Exclusion criteria for the study were as follows: (1) contraindications for the applied techniques, including tDCS and Magnetic Resonance Imaging (MRI), (2) habitual smoking (more than 10 cigarettes per day) or abuse/dependence on other drugs, (3) pregnancy, (4) use of psychoactive drugs, including antidepressant drugs, benzodiazepines, and Z-drugs, and (5) serious clinical conditions. The participants included in the study were contacted from a pool of participants who have previously undergone other studies conducted by our laboratory. Prior to participation, all participants provided written informed consent.

The required sample size for this study was determined by employing a small to moderate effect size of f = 0.25, alpha of 0.05, and a power of 0.80, based on three repeated measurements (G*Power 3.1 software), which generated a required sample size of 28 participants. To account for potential dropouts or data loss, a total of 40 participants were recruited.

Experiment Procedure

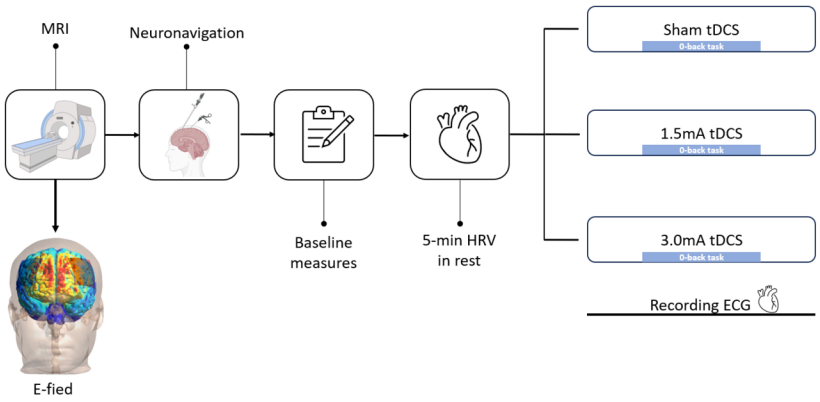
The experiment consisted of four separate visits. Firstly, an anatomical 3-Tesla MRI acquisition was performed (T1- and T2-weighted sequences; repetition time (TR) = 1900 milliseconds, echo time (TE) = 2.2 milliseconds, flip angle = 9°, 176 slices/volume, slice thickness = 0.8 mm). These images were posteriorly used for neuronavigation and for electric field modeling analyses. Afterwards, participants underwent three tDCS experiment sessions (Figure 1).

All experiment sessions took place in a well-controlled laboratory environment. Participants were asked to

sleep sufficiently, and abstain from intense physical activity and alcohol/caffeine consumption 2 hours prior to the session. Throughout all three sessions, participants were seated in a comfortable chair and positioned their knees at a 90-degree angle. In the first experiment session, a neuronavigation procedure (Brainsight, Rogue Resolutions, Inc) based upon the individual subject's MRI image was performed to individually determine the targeted DLPFCs. These targets were marked in a cap that was used in the following sessions.

At the beginning of each session, self-report baseline measurements were collected and the physiological equipment to record cardiac activity was set up. Cardiac activity was then collected for 5 minutes at rest (i.e. baseline period). The neuromodulation session started after the end of the baseline period. A 0-back task was performed together with the tDCS to control and standardize ongoing neural activity during tDCS. For the 0-back, random words appeared on a computer screen and the participant had to press a specific letter on the keyboard of the occurrence of a specific word on the screen. Cardiac activity was collected during the entire tDCS session (20 minutes) (Figure 1).

Figure 1. Study Design



Abbreviation: ECG: Electrocardiogram; E-Field: Electric Field; HRV: Heart Rate Variability; MRI: Magnetic resonance Imaging; tDCS: transcranial direct current stimulation.

Heart rate variability

Cardiac activity was measured continuously and then separated in four time-points: 5-min of baseline (baseline), initial 5 minutes of the tDCS session (5-min tDCS), 10-min during the 0-back task (during 0-back) and the last 5-min of the tDCS session (last 5-min tDCS). Cardiac activity was acquired using a Biopac ECG100C amplifier (Biopac Systems Inc., USA) and Biopac Acqknowledge software 4.3. For the amplifier, the gain was set at 5000, with a high pass filter of 0.05 Hz and a low pass filter of 35 Hz. The sampling rate was set at 1000 Hz. The electrocardiogram was set-up using the lead I configuration (Francis, 2016), with two Ag/Agcl electrodes attached below the right and left clavicle and a third reference electrode placed under the left ribs. The electrocardiogram data were analyzed with PhysioData Toolbox (version 0.6.3) which allows for automated R-peak detection and inter-beat-interval (IBI) extraction. Misidentified R-peaks were manually corrected after visual inspection of the data.

HRV (in milliseconds) was assessed by calculating the Root Mean Squared of Successive Differences (RMSSD) of the detrended IBI data (Tarvainen et al., 2002)and High-Frequency-HRV (HF-HRV, 0.15 to 0.40 Hz), using the absolute power of the HF band, as calculated using the Lomb-Scargle method. Importantly, although there are several ways to investigate HRV, only RMSSD and HF-HRV were analyzed here because they present robust effects when using NIBS intervention over the PFC and they are markers of the parasympathetic system (Makovac et al., 2017).

RMSSD and HF-HRV were calculated for time epochs of 5 min, as recommended elsewhere (Malik et al., 1996).Therefore, for the time-point in which we recorded the HRV during the 0-back task (10min), the average of two epochs of 5 min was obtained.

tDCS

TDCS was performed using a NeuroConn device (DC-Stimulator Plus, NeuroConn, Germany). The session consisted of a 20-minute direct current, delivered using two rubber electrodes of 25cm^2 (5x5cm) applied to the scalp with a conductive gel. The anode and cathode were placed over the left and right neuronavigated DLPFC, respectively (left: x = -38, y = +44, y = +26; right: x = 38, y = +44, y = +26) (Blumberger et al., 2018), pointing towards Cz. Stimulation at these coordinates has been found to achieve optimal anticorrelation with the ACC in functional brain studies (Fox et al., 2012)(Razza et al., 2022). The active tDCS sessions were performed using currents of 1.5mA and 3mA. The sham protocol was identical but consisted of a brief active period of 30 seconds fade-in and 30 seconds fade-out at the beginning and end of the session to a current intensity of 3mA with the device set to 0mA for the remainder. The 'study mode' of the tDCS device was used to deliver active or sham current based on a randomized imputed code, allowing for double-blinding of participants and study personnel. The codes were randomized via*https://www.randomizer.org*and were managed by an independent party not directly involved in data acquisition.

Self-reported measures

The State-Trait-Anxiety Inventory (STAI) state inventory was administered at the beginning of each session (Spielberger et al., 1970). The STAI state measures how participants are feeling at that moment using a 4-point Likert scale (1 = not at all, 2 = a little, 3 = moderately, 4 = very much). This measurement was collected to identify the baseline psychological state of each participant before the tDCS session. Moreover, the Visual Analogue Scale (VAS) was collected two times (at baseline and after the tDCS session). In VAS participants are asked to indicate from 'zero' to 'one-hundred' ("I do not experience this at all", "I experience this very much") how much they were feeling the following moods: 'angry', 'tense', 'sad' 'happy', 'stressed' and 'anxious'. Higher scores indicate higher levels of perceived stress or negative affect.

Computational Modeling Analysis

E-field simulation was performed using SimNIBS (Version 4.0, Copenhagen, Denmark) (Saturnino, Madsen, and Thielscher 2019), an open-source software that allows the estimation of the tDCS-induced E-field distribution in individual brains. Firstly, head models of each participant were generated using *charm* (from individual T1- and T2-weighted structural MRI data (Puonti et al., 2020)), which segments the MRI images into nine distinct tissue types. For this analysis, the default conductivity values for each tissue were applied. Subsequently, the software generated a 3D tetrahedral mesh structure for each segmented tissue, allowing the simulation of the tDCS-induced E-field in each participant's brain. Segmentations were manually verified to investigate any potential errors.

The tetrahedral head meshes resulting from the segmentation procedure were used to simulate the E-field distribution resulting from the two active tDCS protocols (1.5 mA and 3 mA). The sham protocol was not taken into consideration for our analysis as it does not produce an E-field in the head. To simulate the E-field in the brain, the tDCS set-up applied was identical as used in the experimental sessions. The electrodes (5x5cm) were placed over the MNI coordinates retrieved from neuronavigation, pointing towards Cz, with a thickness of 5 mm (electrode + conductivity paste). SimNIBS scripts were executed in MATLAB (version R2022a).

Finally, the E-field magnitude within predefined brain regions of interest were extracted. These regions were the bilateral DLPFC, bilateral ACC, bilateral insula and bilateral amygdala. The DLPFC was extracted from the Sallet atlas (Sallet et al., 2013), whereas the ACC, insula and amygdala were extracted from the Brainnetome atlas (Fan et al., 2016) (Sup. Material - Appendix 1).

Values analyzed in this study were the mean magnitude of the electric field (magn-E component).

Statistical Analysis

All statistical analyses were performed with R software (version 4.1.2, R Core Team,*2021*). To investigate tDCS-induced changes in HRV, we calculated the change from baseline to the tDCS periods (5-min tDCS, during 0-back, and last 5-min tDCS) using delta scores (i.e: Δ score = RMSSD of 5-min tDCS minus RMSSD of baseline or HF-HRV of 5-min tDCS minus HF-HRV of baseline, etc). Outliers were inspected via boxplot distribution and three observations were excluded for the RMSSD data, while none was excluded for the HF-HRV (Sup. Material - Appendix 2). A linear mixed model (LMM) ('lme4' package) was used to assess the effects of tDCS on the HRV. The model included either delta RMSSD or HF-HRV scores as the dependent variable, with a fixed effect of tDCS protocol, time and their interaction (tDCS protocol*time). 'Subject' was employed as a random intercept (full statistical model: 'RMSSD/HF-HRV ~ tDCS protocol*time + (1|Subject))'. Pairwise analyses were performed using the 'emmeans' function.

Secondly, we investigated whether the HRV changes were associated with E-field magnitude induced by tDCS in the brain regions of interest. In the first step we employed LMM models having the RMSSD/HF-HRV delta scores as dependent variables, whereas the mean E-field in each region of interest were the fixed factors. 'Subject' was considered a random intercept. To investigate whether the mean individual E-fields were associated with the outcome, the same model was used but the variable 'protocol' was included as a fixed factor (RMSSD/HF-HRV ~E-field + tDCS protocol + (1|Subject)). In total, eight models were performed per analysis (4 brain regions of interest x 2 hemispheres). Therefore, multiple comparison corrections were conducted using the false discovery rate ('stats' package). Only the corrected p-values are presented here.

Exploratory analyses investigated whether the HRV changes could be influenced by baseline mood or state anxiety. For this analysis a LMM was fitted with the RMSSD/HF-HRV as the dependent variable and the interaction between mood and tDCS protocol (i.e: VAS baseline*tDCS protocol and STAI*tDCS protocol) as the independent variable (full model: RMSSD/HF-HRV ~ mood baseline*tDCS protocol + (1|Subject)). For all statistical tests, the significance level was set to alpha = 0.05.

Results

A total of 40 healthy volunteers were included. Two dropped-out after the first session and one was excluded due to non-normal alterations in the heart rate as indicated by a cardiologist. The final analyses were performed with 37 subjects (mean age = 24.3 years, standard deviation (SD) = 4.8), representing a total of 111 sessions being performed. Due to electrocardiogram artifacts during data collection (especially during the 0-back) a few time points were dropped from our dataset. Therefore, our analyses were conducted with a total of 103 time-points each for sham and 1.5mA, and 96 time-points for the 3.0mA protocol. For more information, a comprehensive table can be found in the Sup. Material - Appendix 3.

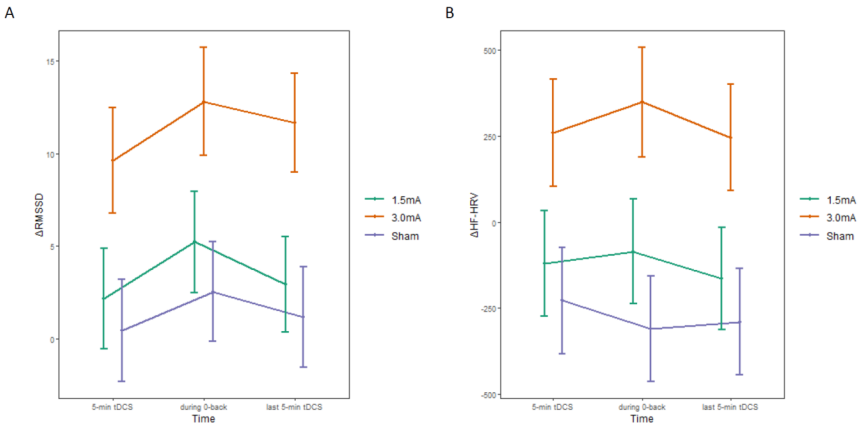
tDCS protocol and HRV

The results of our analysis revealed a significant effect of tDCS protocol (chi-square ($\chi2$) = 8.02, p = 0.018) in the RMSSD measure. Post-hoc analyses revealed that HRV during 3.0mA tDCS, measured with RMSSD, was significantly increased compared to sham (beta= -9.97, standard error (SE) = 1.96, p < 0.001) and 1.5mA (beta= -7.92, SE = 1.94, p = 0.001). No significant difference was found between 1.5mA tDCS and sham (beta= -2.06, SE = 1.9, p = 0.28) (Figure 2A and Sup. Material - Appendix 4). The interaction between tDCS protocol and time is presented in the Sup. Material - Appendix 5.

The same results were found for the HF-HRV measure (tDCS protocol: $\chi2$ = 6.88, p = 0.03). Post-hoc analyses revealed that HF-HRV were greater with 3.0mA compared to sham (beta= -606, SE = 112, p < 0.001) and 1.5mA (beta= -454, SE = 112, p = 0.001) (Figure 2B and Sup. Material - Appendix 6). The interaction between tDCS protocol and time is presented in the Sup. Material - Appendix 7.

No association was found between HRV changes and the interaction between protocol and psychological state at the baseline (STAI: $\chi2$ = 2.18, p = 0.33; VAS baseline: $\chi2$ = 0.45, p = 0.79).

Figure 2. Effects of tDCS on HRV. A) Results of RMSSD; B) Results of HF-HRV.



HRV and E-field magnitude

A significant association was found between both measures of HRV (RMSSD and HF-HRV) and the E-field magnitude in all brain regions of interest, showing that stronger E-field are able to induce greater HRV changes (Table 1 and Figures 3 and 4).

Table 1. Mixed effects model outcomes showing the association between E-field strength in the brain regions of interest and HRV modulation.

RMSSD	**RMSSD Left**	**RMSSD Left**	**RMSSD Left**	**RMSSD Left**	**RMSSD**	**RMSSD Right**	**RMSSD Right**	**RMSSD Right**	**RMSS Right**
	Beta	SE	t-value	p-value		Beta	SE	t-value	p-valu
DLPFC	37.54	8.6	4.36	<0.001		35.54	8.8	4	<0.00
ACC	48.3	11.4	4.2	<0.001		51.1	12.7	4	<0.00
Amygdala	52.2	12.9	4.03	<0.001		52.6	13.6	3.9	<0.00
Insula	53.3	12.8	4.16	<0.001		48.7	12.5	3.9	<0.00
HF-HRV	**HF-HRV Left**	**HF-HRV Left**	**HF-HRV Left**	**HF-HRV Left**	**HF-HRV**	**HF-HRV Right**	**HF-HRV Right**	**HF-HRV Right**	**HF-H Right**
	Beta	SE	t-value	p-value		Beta	SE	t-value	p-valu
DLPFC	2063	482	4.3	<0.001		1986	496	4	<0.00
ACC	2696	640	4.2	<0.001		2891	714	4.1	<0.00
Amygdala	2994.5	725.7	4.12	<0.001		3054.5	764	4	<0.00
Insula	3020	721	4.18	<0.001		2820	708	3.9	<0.00

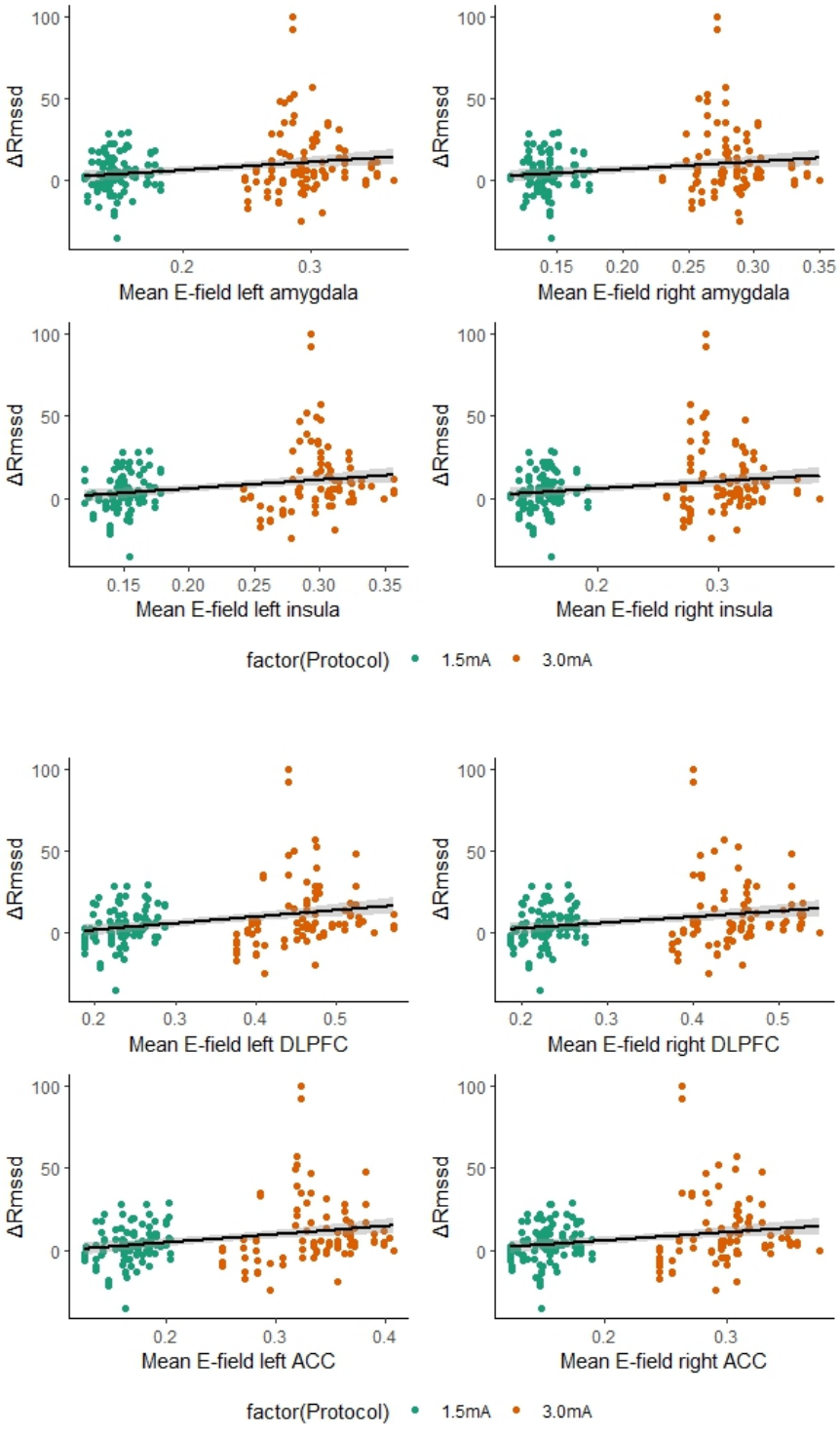
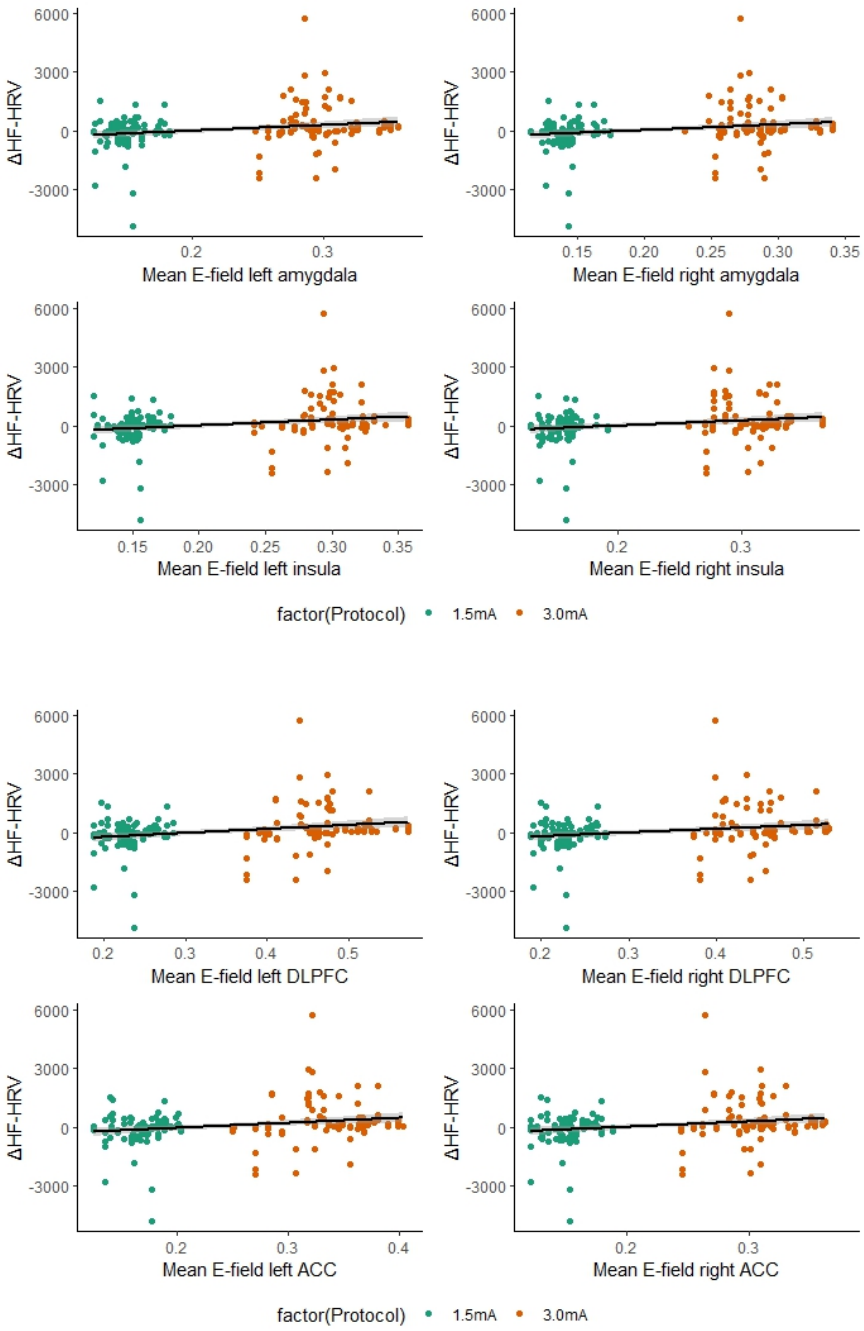


Figure 3. Association between RMSSD and E-field in all brain regions of interest.

Figure 4. Association between HF-HRV and E-field in all brian regions of interest.



In turn, no significance was found when including *tDCS protocol*in the model, showing that changes in HRV might not be associated with individual E-field variability in brain regions of interest (Table 2). E-field head models are presented in the Sup. Material - Appendix 8.

Table 2. Mixed effects model outcomes controlled by 'tDCS protocol' showing no association between E-field strength in the brain regions of interest and HRV modulation.

RMSSD	**RMSSD Left**	**RMSSD Left**	**RMSSD Left**	**RMSSD Left**	**RMSSD**	**RMSSD Right**	**RMSSD Right**	**RMSSD Right**	**RMSS Right**

	Beta	SE	t-value	p-value		Beta	SE	t-value	p-valu
DLPFC	65.9	42.9	1.5	0.13		-0.2	48.2	-0.005	0.99
ACC	59.1	53	1.1	0.26		7.6	67.6	0.1	0.9
Amygdala	0.67	79.3	0.01	0.98		-83.9	86.6	-0.96	0.33
Insula	62.9	83.4	0.75	0.45		-69.3	83.6	-0.8	0.4
HF-HRV	**HF-HRV Left**	**HF-HRV Left**	**HF-HRV Left**	**HF-HRV Left**	**HF-HRV**	**HF-HRV Right**	**HF-HRV Right**	**HF-HRV Right**	**HF-H Right**
	Beta	SE	t-value	p-value		Beta	SE	t-value	p-valu
DLPFC	3350	542	-0.57	0.56		361	2610	0.14	0.89
ACC	2892	2876	1	0.32		935	3621	0.26	0.79
Amygdala	2596	4204	0.62	0.53		-648	4746	-0.14	0.89
Insula	4458	4584	0.97	0.33		-1499	4745	-0.31	0.75

Discussion

This study aimed to investigate the modulatory effects of distinct prefrontal tDCS intensities (1.5mA, 3mA and sham) on HRV and explored whether the magnitude of the E-field in brain regions of interest was associated with this outcome. Our hypotheses posited that higher electric currents would lead to increased HRV, while individual anatomical variability would also play a significant role in this modulation. Specifically, we expected that individuals with higher magnitudes of E–fields in brain regions of interest would exhibit greater increases in HRV, as measured by RMSSD and HF-HRV. As hypothesized, the results showed that tDCS was able to modulate both cardiovascular measures via a top-down route. However, only the highest electric intensity (of 3.0mA) increased HRV compared to sham and 1.5mA current. According to our findings, this modulation was not associated with anatomical individual differences per se, as evaluated by computational modeling analysis.

Although this study did not provide evidence to support our hypothesis that inter-individual variability contributes to the heterogeneous effects of tDCS, the results presented here are aligned with the dose-dependent effects of tDCS - with higher electric current intensities producing increased RMSSD and HF-HRV (Goldsworthy & Hordacre, 2017). In this context, a recent meta-analysis showed that the effects of prefrontal tDCS might be only small to moderate for both RMSSD and HF-HRV measures of healthy subjects (Schmaußer et al., 2022). However, it is important to note that a limitation of the aforementioned study was that potential moderators of response to tDCS were not investigated, including tDCS protocols. Therefore, our findings suggest, for the first time, that the variability of tDCS effects on cardiovascular measures might be associated with the heterogeneity of tDCS protocol (as different electric current is applied across published studies, i.e: 1mA, 1.5mA and 2mA), rather than inter-individual anatomical variability.

Following the central autonomic network model, the brain-body connection is important for regulating parasympathetic control and autonomic balance (Cameron, 2009). This occurs when the modulation of cortical and subcortical brain regions - such as the ones discussed here - has the potential to activate parts of the autonomic nervous system that can regulate oscillations of the heart rate (Mulcahy et al., 2019). Hence, it's important to ensure that this top-down approach (from PFC, to subcortical areas to autonomic nervous system) using tDCS and other non-invasive brain stimulation interventions seems effective. As tDCS delivers a low electric current into the brain and almost 75% of this current is deflected by different layers including skin, bone, hair and cerebrospinal fluid, only a small percentage of the current is indeed able to reach cortical tissue (Vöröslakos et al., 2018). In this sense, we believe that higher currents (i.e: 3mA), compared to lower currents (i.e: 1.5mA) are better able to penetrate into the (sub-) cortical regions, and thereby efficiently modulate the PFC as well as the parasympathetic system via a top-down regulation.

Moreover, it is worth mentioning that the present study employed a neuronavigation method aiming to accurately target the MNI coordinate in the DLPFC optimally associated with the subgenual ACC in depressed patients, using transcranial magnetic stimulation (Fox et al., 2012). Although the tDCS electrodes are larger (5x5cm), the utilization of this precise targeting approach might have increased the connection

between PFC and subcortical areas. This is important to note, as previous studies used target location based on the 10-20 EEG system or Beam-F3, both of which are valid methods in the field, but are less accurate than neuronavigation. Therefore, this approach should be suggested for future researchers evaluating tDCS on HRV of depressed patients.

Although our study did not reveal significant inter-individual differences, the analysis utilizing E-field modeling yielded two important findings. Firstly, all the brain regions of interest exhibited associations with the outcome measure, indicating that greater simulated electric current in these areas was associated with more effective manipulation of HRV. This finding further supports the notion that when prefrontal tDCS is applied to healthy individuals, it impacts and modulates all regions that are correlated with the central autonomic network. Secondly, the graphical representation of the E-field results indicates that the administration of a higher current intensity (3.0mA) is associated with increased variability in the E-field within the brain regions of interest. This observation suggests a greater potential for modulatory effects and room for improvement when higher currents are applied.

Both ours and Nikolin and colleagues (Nikolin et al., 2017) studies showed overall increased HF-HRV for active tDCS conditions relative to sham. Overall, the results of the 1.5mA protocol of our study seems really close to what they presented using a current of 2mA. This comparison also supports our hypothesis of a dose-dependent relationship. Finally, our study did not demonstrate a reduction in HRV measures during the concurrent cognitive task when combined with tDCS, as seen by Nikolin and colleagues (Nikolin et al., 2017) . In fact, the graphical representations visually depicted an increase in parasympathetic effects during the 0-back performance, although this finding did not reach statistical significance. It is important to note that while HRV measures typically decrease in stressful situations, the individuals in our study were exposed to an attentional task that engaged PFC activity, which is known to increase HRV. Thus, the engagement of PFC activity during the attentional task may have contributed to the observed increase in HRV, despite the absence of statistical significance.

Limitation

While this study has several strengths, such as a well-powered within-subjects design and the measurement of various parasympathetic indicators, it is important to acknowledge and address some notable limitations. First, a measure of the sympathetic activity was not evaluated. Therefore, we were not able to investigate how tDCS, via increasing parasympathetic control, can affect the sympathetic nervous system. Second, the neuronavigation method used here was based on a predetermined MRI coordinate. Perhaps, applying individualized DLPFC location based on functional MRI targeting (Cash et al., 2021) may be a further improvement for future studies. Third, due to the within-subject design, blinding was not assessed as it could increase participants' awareness applying individualized DLPFC location regarding the intervention protocol in the subsequent session.

Conclusion

This study examined the modulatory effects of different tDCS intensities on HRV in healthy individuals. The findings support the dose-dependent effects of tDCS, with the highest electric current intensity (3.0mA) demonstrating a significant increase in HRV compared to both sham and 1.5mA current. While the individual amount of electric current penetrating the brain did not appear to significantly influence the tDCS effects on HRV modulation, E-field modeling analysis suggests that the heterogeneity of tDCS protocols may contribute to the variability in tDCS effects on cardiovascular measures. This study highlights the effects of tDCS on parasympathetic activity of healthy subjects via prefrontal tDCS, showing a dose-dependent effect. Based on our results, we believe that further research is warranted to investigate the optimal tDCS parameters for HRV modulation.

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