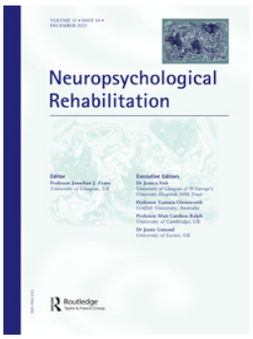

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High definition tDCS of the left dorsolateral prefrontal cortex modulates nonlinear HRV in high test anxiety individuals

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ABSTRACT

Prior neurostimulation research has underscored the importance of the left dorsolateral prefrontal cortex (DLPFC) in the modulation of autonomic nervous system responses, with activation of this region significantly enhancing heart rate variability (HRV) – a key indicator of autonomic flexibility. High test anxiety (HTA) individuals often demonstrate diminished HRV in evaluative scenarios, signalling reduced physiological adaptability to stress. This study investigates the effects of offline high-definition transcranial direct current stimulation (HD-tDCS) targeting the left DLPFC on nonlinear HRV measures in individuals with high levels of test anxiety. Two experiments were conducted: Experiment 1 measured HRV in 23 HTA participants before and after HD-tDCS application. Experiment 2 replicated the tDCS protocol with 24 participants with low test anxiety (LTA). Significant enhancement in nonlinear HRV post-HD-tDCS was observed in HTA group, whereas no notable changes were reported in HRV among LTA group. These results indicate that HD-tDCS targeting the left DLPFC may be an effective intervention for managing test anxiety, as it appears to improve autonomic regulation in individuals experiencing high anxiety levels.

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Test anxiety; transcranial direct current stimulation; left dorsolateral prefrontal cortex; heart rate variability

Introduction

Test anxiety refers to individuals' physiological and behavioural responses in evaluative contexts, accompanied by concerns about potential adverse outcomes or failure (Zeidner, 1998). In the past 15 years, the average occurrence rate of test anxiety among Chinese students has reached 22.32%. Despite

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rates of test anxiety remaining relatively stable at around 30% in junior and senior high school students, a steady increase has been observed among university students (Huang & Zhou, 2019). Test anxiety not only negatively predicts students' subjective well-being (Steinmayr et al., 2016) but also impairs their executive functions and academic performance (O'Donnell, 2017; Song et al., 2021; von der Embse et al., 2018; Wei et al., 2022). Uncovering the neurophysiological mechanisms of test anxiety can provide a basis for effective interventions to address this issue.

Heart rate variability (HRV) represents the degree of variation in the time intervals between heartbeats (Mccraty & Shaffer, 2015). It serves as both an indicator of cardiac health and a measure of the integration between the central nervous system and the autonomic nervous system, reflecting an individual's adaptability and responsiveness to external stimuli (Thayer et al., 2012). From the perspective of the neurovisceral integration model, the default response to stress and threat in humans involves sympathetic nervous system activation, often referred to as the "fight or flight" response (Thayer & Lane, 2009). The prefrontal cortex plays a pivotal role in evaluating threats and determining the appropriateness and timing of this "fight or flight" response to prevent prolonged stress and the resulting allostatic load (McEwen, 1998). Such prolonged stress can lead to anxiety (Thayer et al., 2012), with HRV serving as an index of this integrated system.

However, test anxiety can exacerbate an individual's stress response in evaluative situations, resulting in heightened sympathetic nervous system activity, manifested as increased heart rate and elevated blood pressure (Roos et al., 2021). Additionally, test anxiety is associated with a decrease in high-frequency HRV and the root mean square of the successive differences (RMMSD) (Bian et al., 2022). According to the neurovisceral integration model, individuals with test anxiety may perceive tests as threats, leading to an inability to suppress sympathetic nervous system activation. This ongoing activation can ultimately lead to a close coupling between the central nervous system and the autonomic nervous system, making it easier to trigger threat responses and, consequently, reduce HRV. However, there has been no direct investigation confirming the relationship between the prefrontal cortex and HRV in individuals with test anxiety.

Non-invasive brain stimulation (NIBS) involves the use of external devices placed on the head to modulate the excitability of cortical neurons, providing insights into the causal relationships between brain regions and cognitive functions, autonomic responses, and more (Zhou et al., 2023; Ziemann et al., 2008). The most commonly utilized NIBS techniques include transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS). In the context of the relationship between the prefrontal cortex and HRV, the left DLPFC serves as a critical intervention site. Remue et al. (2016) demonstrated that high-frequency rTMS applied to the left DLPFC significantly increased the

RMMSD in healthy adults under stress conditions. Brunoni et al. (2013) found that anodal tDCS targeting the left DLPFC significantly enhanced high-frequency HRV in healthy adults while viewing negative images. De Putter et al. (2015) combined working memory training with anodal tDCS applied to the left DLPFC, resulting in increased high-frequency HRV in resting state among healthy adults. In the context of the neurovisceral integration model, DLPFC serves as a critical hub for top-down control (Thayer et al., 2012). Crucially, individuals with high test anxiety often exhibit deficits in this top-down regulation. Resting-state fMRI studies have revealed that individuals with test anxiety show reduced activity and functional connectivity within the Executive Control Network, particularly in prefrontal regions (Hou et al., 2022; Huang et al., 2022). Furthermore, anxiety is frequently associated with an inter-hemispheric imbalance, characterized by hypoactivity in the left DLPFC and hyperactivity in the right DLPFC (Grimm et al., 2008; Nitschke & Heller, 2005). Consequently, enhancing the excitability of the left DLPFC via anodal tDCS may strengthen the inhibitory control over subcortical autonomic centres, thereby improving HRV among high test anxiety individuals. However, the role of the left DLPFC in HRV in individuals with test anxiety remains unclear. Therefore, this study aims to employ anodal high-definition tDCS(HD-tDCS) to investigate the effects of the left DLPFC on HRV in individuals with high test anxiety.

In recent years, nonlinear indices of HRV have been widely employed in the diagnosis and analysis of diseases and mood disorders, and their efficacy surpasses that of time-domain and frequency-domain analysis methods (Greco et al., 2018; Faust et al., 2012). Nonlinear indices reflect the complexity of HRV, aligning with the contemporary organismic perspective based on complexity theory, which posits that the stability, adaptability, and health of organisms are maintained through the variability of dynamic relationships among system elements (Thayer & Sternberg, 2006). Consequently, an increase in nonlinear HRV may foreshadow a healthier physiological and emotional state in individuals. Among the nonlinear indices of HRV, correlation dimension (D_2) is considered a marker of cardiovascular system adaptability (Delliaux et al., 2019). D_2 quantifies the temporal self-similarity of signals and is commonly employed as an indicator of overall complexity in the estimation of system dynamics from time series data (Sammer, 1998). In this study, D_2 is also utilized as a metric to assess the enhancement of nonlinear HRV in individuals with high test anxiety following HD-tDCS.

Based on existing research, this study aims to investigate whether HD-tDCS targeting the left DLPFC can enhance nonlinear HRV in individuals with high test anxiety. Experiment 1 will involve participants with high test anxiety (HTA), and it is hypothesized that anodal HD-tDCS applied to the left DLPFC will significantly increase D_2 in HTA individuals. To further assess the specificity of this intervention for HTA individuals, Experiment 2 will replicate Experiment 1 using participants with low test anxiety (LTA). See Figure 1 for the overall flowchart.

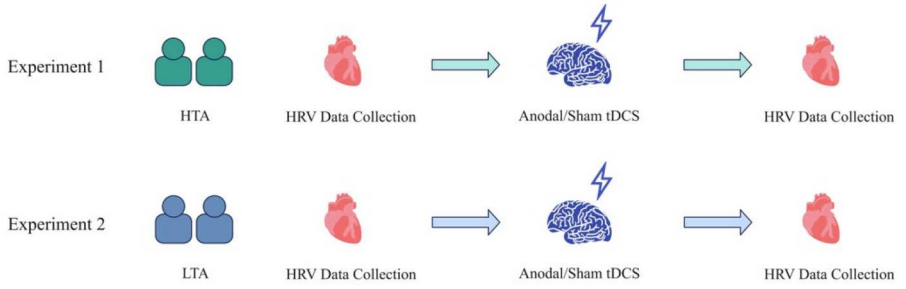

Figure 1. Overview of the experimental procedure flowchart.

Two experimental flow diagrams compare high and low test anxiety groups. Both show HRV measurement before and after either anodal or sham tDCS applied over the left dorsolateral prefrontal cortex.

The figure presents two experimental sequences. In Experiment 1, high test anxiety participants are depicted as two green human icons. A red heart icon indicates heart rate variability (HRV) data collection before stimulation. An arrow points to a blue brain with a lightning bolt, representing either anodal or sham high-definition transcranial direct current stimulation (tDCS) applied to the left dorsolateral prefrontal cortex. A second arrow points to another red heart icon, indicating HRV data collection after stimulation. Experiment 2 follows the same sequence with low test anxiety participants, represented by two blue human icons. The flow similarly shows HRV data collection before and after tDCS or sham stimulation, highlighting the parallel experimental design across both anxiety groups.

Experiment 1: The effect of left DLPFC on nonlinear HRV in HTA individuals

Method

Participants

Participants in this study were undergraduate and graduate students from Nanjing University. Participants were selected based on their scores on the Test Anxiety Scale (TAS) (Newman, 1996). According to the criteria, individuals with TAS scores exceeding 20 were considered to have high test anxiety and were included in this experiment. To control for the potential influence of depression, participants were also required to score below 13 on the Beck Depression Inventory (BDI) to be eligible (Beck et al., 1996). A total of 23 participants were included in this experiment, comprising 9 females and 14 males, with ages ranging from 19 to 24 years, with a mean age of 21.74 years (SD = 1.81). A post-hoc analysis was conducted using G*Power 3.1(Faul et al., 2009), with the effect size set at 0.25 and an alpha level of 0.05. It was found that a sample size of 23 participants could achieve a statistical power of 0.80. All participants had normal or

corrected-to-normal vision, were right-handed, and reported no history of epilepsy, traumatic brain injury, or psychiatric disorders. This experiment was approved by the Ethics Committee of the Department of Psychology at Nanjing University. All participants volunteered to participate, provided informed consent before the experiment, and received compensation upon completion of the study.

Experimental design and procedure

Experiment 1 was designed as a single-blind, within-subjects, sham-controlled trial on two separate days. Each subject received true or sham tDCS intervention on two different days with a 3-day washout. Experiment 1 employed a 2 (Stimulation Type: Anodal vs. Sham) x 2 (Time: Pre-test vs. Post-test) experimental design, with the dependent variable being the nonlinear HRV index, D_2. Prior to and following each tDCS intervention, 8 min of resting-state HRV data were collected.

Transcranial direct current stimulation

HD-tDCS was applied using five high-definition electrodes (12 mm external diameter; Soterix Medical®, New York, NY, USA). Stimulation electrodes were placed in a 4×1 montage over right prefrontal sites (with 4 surrounding +1 central electrodes forming a closed circuit). A total of five high-definition electrodes were carefully positioned over the left DLPFC. The central electrode was precisely located at site F3, while the four surrounding electrodes were strategically positioned at F1, FC3, F5, and AF3, according to the 10–20 international system for EEG electrode placement (Feeser et al., 2014). Throughout the stimulation session, every impedance was checked to ensure that electrode impedance remained at or below 5 kΩ. Under the anodal stimulation condition, participants received a constant current of 1.5 mA for a duration of 20 min, with a 30s fade-in at the start and a 30s fade-out at the end of the stimulation. In the sham stimulation condition, stimulation consisted solely of a 30s fade-in at the initiation and a 30s fade-out at the conclusion, with the instrument automatically ceasing stimulation for the remainder of the session. To ensure unbiased results, the order of anodal and sham tDCS was counterbalanced across all participants, who remained unaware of the specific stimulation condition they received during each session. To verify the success of blinding, participants rated the intensity of physical sensations (itching, tingling, pain, burning, and fatigue) on a 10-point scale at the end of the experiment. Paired t-tests revealed no significant differences in the intensity of reported sensations between the anodal and sham conditions (all $ps > .05$).

HRV data collection and analysis

The participants' electrocardiogram (ECG) data were collected using the ECG100C amplifier of the BIOPAC MP160 multi-channel physiological recording

system, with a sampling rate of 1000 Hz. Data were filtered using a high-pass filter of 0.5 Hz and a low-pass filter of 35 Hz. Three disposable Ag-AgCL electrodes were placed on the participants' wrists and ankle, with the left wrist connected to the positive electrode, the right wrist connected to the negative electrode, and the right ankle connected to the ground electrode. HRV data were simultaneously recorded using AcqKnowledge 4.2 software. The HRV data were processed using Kubios HRV Scientific 4.0.1 (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland), which included R-wave detection, automatic correction, and calculation of the nonlinear index D_2. Kubios HRV preprocessing settings were at the default values including the RR detrending method which was kept at "Smoothn priors" (Lambda = 500) (Tarvainen et al., 2014). The computation method for D_2 was in accordance with Faust and Bairy (2012).

Results

Descriptive statistics for D_2 values under the four conditions are presented in Table 1. Repeated measures analysis of variance (ANOVA) with a 2 (Stimulation Type: Anodal vs. Sham) x 2 (Time: Pre-tDCS vs. Post-tDCS) design was conducted using SPSS 26.0. As shown in Figure 2, the main effect of Stimulation Type was not significant, $F(1, 22) = 1.48$, $p = .236$, $\eta_p^2 = 0.063$. The main effect of Time was significant, $F(1, 22) = 9.87$, $p = .005$, $\eta_p^2 = 0.31$, with Post-test D_2 values significantly higher than Pre-test values. There was also a significant interaction between Stimulation Type and Time, $F(1, 22) = 5.34$, $p = .031$, $\eta_p^2 = 0.195$. Simple effects analysis revealed that under the anodal stimulation condition, Post-tDCS D_2 values were significantly higher than Pre-tDCS values, $F(1, 22) = 13.41$, $p = .001$, $\eta_p^2 = 0.379$. However, under the Sham Stimulation condition, there was no significant difference between Pre-tDCS and Post-tDCS D_2 values, $F(1, 22) = 1.62$, $p = .217$, $\eta_p^2 = 0.068$. In addition, simple effects analysis also indicated that there was no significant difference between anodal and sham stimulation conditions at Pre-tDCS, $F(1, 22) = 3.79$, $p = .064$, $\eta_p^2 = 0.147$. Likewise, at Post-tDCS there was no significant difference between the anodal and sham stimulation conditions, $F(1, 22) = 0.33$, $p = .572$, $\eta_p^2 = 0.015$.

Table 1. Descriptive statistics (Mean ± SD) and ANOVA results for cardiac measures in Experiment 1 (HTA group, $n = 23$).

Measure	Condition	Pre-tDCS	Post-tDCS	Interaction effect
D_2	Anodal	2.22 ± 1.49	3.30 ± 1.09	$F = 5.34$, $p = .031$
	Sham	2.85 ± 1.48	3.18 ± 1.23	
Heart Rate (bpm)	Anodal	77.00 ± 10.00	70.20 ± 7.97	$F = 0.14$, $p = .709$
	Sham	75.10 ± 10.30	68.80 ± 9.05	
RMSSD (ms)	Anodal	44.20 ± 26.00	56.30 ± 39.00	$F = 0.07$, $p = .796$
	Sham	45.30 ± 21.80	59.30 ± 35.60	
HF (ms^2)	Anodal	943 ± 1081	1277 ± 1415	$F = 2.57$, $p = .123$
	Sham	993 ± 976	1788 ± 2252	

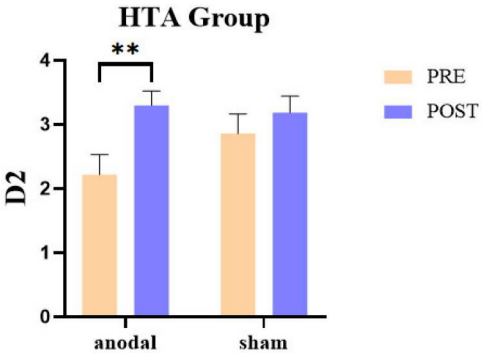

Figure 2. D_2 values under four conditions in Experiment 1. Note: $^{**}p < .01$.

In addition to D_2, we analysed standard linear cardiac measures including Heart Rate (HR), RMSSD, and High-Frequency power (HF). As shown in Table 1, repeated measures ANOVA revealed no significant Stimulation × Time interaction effects. These null results on linear indices underscore that the anodal HD-tDCS specifically modulated the nonlinear complexity (D_2) of the autonomic nervous system.

Bar chart showing D_2 index in the high test anxiety group. Anodal tDCS significantly increased post-stimulation D_2 compared to pre, while sham stimulation showed no change.

The figure presents a bar chart of D_2 index values for high test anxiety participants. The y-axis ranges from 0 to 4, labelled D_2. The x-axis has two stimulation conditions: anodal and sham. For the anodal condition, the pre-stimulation bar (orange) is around 2.2, while the post-stimulation bar (blue) is approximately 3.4, with a significant increase indicated by a double asterisk above the comparison bracket. For the sham condition, the pre-stimulation bar is near 2.9 and the post-stimulation bar is near 3.2, showing no significant difference. Error bars are displayed for all bars. The legend indicates orange as PRE and blue as POST.

Discussion

The results of Experiment 1 demonstrate that only under the condition of anodal stimulation, the Post-tDCS D_2 values of HTA group were significantly higher than the Pre-tDCS. This indicates that anodal HD-tDCS targeting the left DLPFC significantly increased D_2 in the participants, validating the role of the left DLPFC in nonlinear HRV within the HTA individuals. However, under the sham stimulation condition, there were no significant changes in D_2 between Pre-tDCS and Post-tDCS, suggesting that in a resting state, HTA individuals may not exhibit autonomous recovery or augmentation in nonlinear HRV.

Experiment 2, which will be a replication of Experiment 1 with LTA individuals, aims to investigate two issues: (1) whether the effect of anodal

stimulation of the left DLPFC on nonlinear HRV is specific to HTA individuals or also present among LTA individuals. This will provide insights into specific intervention targets for test anxiety. (2) We hypothesize that if there are dynamic differences in nonlinear HRV between HTA and LTA group, we may observe different patterns of D_2 changes under the sham stimulation condition in Experiment 2. This comparison will help reveal differences in the dynamic changes of nonlinear HRV between HTA and LTA individuals, further enhancing our understanding of the physiological basis of test anxiety.

Experiment 2: The effect of left DLPFC on nonlinear HRV in LTA individuals

Methods

Participants

Participants in this study were undergraduate and graduate students from Nanjing University. Participants were selected based on their scores on the Test Anxiety Scale (TAS) (Newman, 1996), with individuals scoring <12 on TAS considered to have low test anxiety and eligible for participation. To exclude the influence of depression, only participants with scores below 13 on the Beck Depression Inventory (Beck et al., 1996) were included in this study. A total of 24 participants were included, consisting of 13 females and 11 males, with ages ranging from 19 to 24 years, with an average age of 20.5 years and a standard deviation of 1.35. A post-hoc analysis was conducted using G*Power 3.1(Faul et al., 2009), with the effect size set at 0.25 and an alpha level of 0.05. It was found that a sample size of 24 participants could achieve a statistical power of 0.82. All participants had normal or corrected-to-normal vision, were right-handed, and reported no history of epilepsy, head injuries, or mental illnesses. The experimental protocol was approved by the Ethics Committee of the Department of Psychology at Nanjing University. All participants provided informed consent before the experiment and received appropriate compensation upon completion.

Experimental design and procedure

The experimental design and procedure were identical to Experiment 1.

Transcranial direct current stimulation

The tDCS procedure was the same as in Experiment 1.

HRV data collection and analysis

HRV data collection and analysis followed the same procedures as in Experiment 1.

Results

Descriptive statistics for D_2 values under the four conditions are presented in Table 2. Repeated measures analysis of variance of variance (ANOVA) with a 2 (Stimulation Type: Anodal vs. Sham) x 2 (Time: Pre-tDCS vs. Post-tDCS) design was conducted using SPSS 26.0. As shown in Figure 3, the main effect of Stimulation Type was not significant, $F(1, 23) = 1.29$, $p = .268$, $\eta_p^2 = 0.053$. However, the main effect of Time was significant, $F(1, 23) = 6.97$, $p = .015$, $\eta_p^2 = 0.233$, with Post-tDCS D_2 values significantly higher than Pre-tDCS values. There was no significant interaction between Stimulation Type and Time, $F(1, 23) = 0.16$, $p = .696$, $\eta_p^2 = 0.007$.

Consistent with Experiment 1, we also analysed standard linear cardiac measures (Heart Rate, RMSSD, and HF) for the LTA group. As detailed in Table 2, repeated measures ANOVA revealed no significant Stimulation × Time interaction effects.

Bar chart showing D_2 index in the low test anxiety group. Both anodal and sham tDCS conditions show no significant difference between pre – and post-stimulation.

The figure presents a bar chart of D_2 index values for low test anxiety participants. The y-axis ranges from 0 to 4, labelled D_2. The x-axis has two stimulation conditions: anodal and sham. For the anodal condition, the pre-stimulation bar (orange) is around 2.5, and the post-stimulation bar (blue) is approximately 3.1, showing a slight, non-significant increase. For the sham condition, the pre-stimulation bar is near 2.2 and the post-stimulation bar is close to 2.9, also without significant difference. Error bars are displayed for all bars. The legend indicates orange as PRE and blue as POST.

Discussion

The results of Experiment 2 indicate that there was no significant difference in the increase of D_2 values between Post-tDCS and Pre-tDCS under both anodal and sham stimulation conditions. This suggests that anodal HD-tDCS targeting the left DLPFC does not have an impact on the nonlinear HRV of LTA group.

Table 2. Descriptive statistics (Mean ± SD) and ANOVA results for cardiac measures in Experiment 2 (LTA group, $n = 24$).

Measure	Condition	Pre-tDCS	Post-tDCS	Interaction effect
D_2	Anodal	2.59 ± 1.66	3.15 ± 1.24	$F = 0.16$, $p = .696$
	Sham	2.16 ± 1.84	2.89 ± 1.67	
Heart Rate (bpm)	Anodal	74.30 ± 7.45	68.60 ± 6.18	$F = 1.01$, $p = .325$
	Sham	74.90 ± 12.50	68.20± 10.40	
RMSSD (ms)	Anodal	42.80 ± 23.60	49.90 ± 19.80	$F = 0.98$, $p = .332$
	Sham	43.40 ± 28.90	53.80 ± 31.80	
HF (ms^2)	Anodal	937 ± 1364	971 ± 719	$F = 0.75$, $p = .394$
	Sham	991 ± 1479	1177 ± 1340	

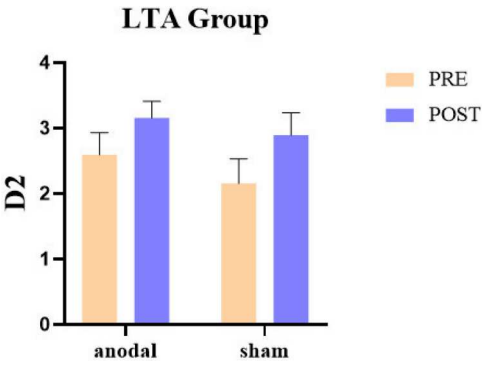

Figure 3. D^2 values under four conditions in Experiment 2.

Additionally, the significant main effect of Time suggests that D_2 in LTA individuals can autonomously recover or increase over time in a resting state. These findings differ from those of Experiment 1, indicating differences in neurovisceral integration between HTA and LTA individuals.

Discussion

This study employed HD-tDCS to manipulate the activation of the left DLPFC and examined its effect on resting-state nonlinear HRV in HTA and LTA individuals. The results indicated that in HTA group, under the condition of anodal stimulation, post-tDCS nonlinear HRV (D_2) significantly increased compared to pre-tDCS, while there was no change under sham stimulation. In contrast, in LTA group, regardless of anodal or sham stimulation, post-tDCS nonlinear HRV was significantly higher than pre-tDCS. It is important to note that no significant difference in baseline D2 values was found between the HTA and LTA groups ($p = .682$). This lack of baseline divergence may reflect a shared initial physiological arousal associated with the laboratory environment. However, the critical distinction between the groups lies in their dynamic adaptation process. The LTA group demonstrated an autonomous recovery of nonlinear HRV over time (a significant main effect of Time), indicative of intact autonomic flexibility that allows for physiological restoration during rest. In contrast, HTA individuals failed to show this natural recovery under sham conditions, exhibiting a deficit in autonomous regulation. The anodal HD-tDCS effectively restored this dynamic regulatory capacity in HTA individuals, enabling them to achieve HRV complexity levels comparable to the post-rest state of the healthy LTA group.

These findings suggest the left DLPFC plays a significant role in the autonomic nervous system complexity of HTA individuals. This study corroborated the role of the left DLPFC in resting-state HRV in the HTA group, which is consistent with the findings of De Putter et al. (2015) and Remue et al. (2016), further confirming the relationship between the prefrontal cortex and HRV

(Thayer et al., 2009). The neurovisceral integration model provides a theoretical basis for the tDCS-induced enhancement of resting-state HRV in HTA individuals. From the perspective of this model, interactions occur between the cerebral cortex and subcortical regions. The prefrontal cortex exerts inhibitory control over subcortical regions and the autonomic nervous system. Resting-state HRV reflects the prefrontal cortex's ability to inhibit subcortical circuits. Consequently, higher levels of resting HRV are associated with better emotional regulation (Williams et al., 2015). In this study, the mechanism underlying the efficacy of Experiment 1 may be that anodal tDCS applied to the left DLPFC activates the prefrontal cortex, enabling it to better inhibit subcortical structures such as the amygdala, thus influencing autonomic nervous system activity and manifesting as higher resting HRV in physiological measures.

Interestingly, while anodal tDCS significantly enhanced nonlinear HRV (D_2), it did not elicit significant interaction effects on standard linear cardiac measures, including Heart Rate, RMSSD, and HF power. This dissociation suggests that the intervention specifically targeted the nonlinear complexity of neurovisceral integration rather than altering gross autonomic tone or baseline heart rate. This finding is consistent with the notion that nonlinear indices may be more sensitive than linear measures in detecting subtle changes in autonomic regulation associated with anxiety and prefrontal modulation (Greco et al., 2018). Furthermore, the stability of heart rate across conditions further corroborates that the observed effects were not driven by physiological arousal resulting from peripheral discomfort or pain.

However, the effect of the left DLPFC on nonlinear HRV was not replicated in Experiment 2 with the LTA group, which aligns with the findings of Jenkins et al. (2002) and Sibon et al. (2007). The differences in experimental results between HTA and LTA groups may be due to distinct physiological states and neural mechanisms. HTA individuals typically exhibit heightened sympathetic nervous system activation, such as increased heart rate and elevated blood pressure (Roos et al., 2021), as well as reduced HRV (Bian et al., 2022). Resting-state fMRI studies have shown that several cortical regions in the executive control network (ECN) and the local gyrification index (LGI) of the right posterior parietal cortex are lower in HTA individuals than in LTA individuals. Furthermore, abnormal resting-state functional connectivity has been observed in the HTA group, including within the ECN, between the ECN and visual network, and between the ECN and sensorimotor network, providing preliminary evidence for alterations in brain morphology and function related to impaired physiological regulation in HTA individuals (Hou et al., 2022). Another fMRI study simultaneously examined functional connectivity in HTA and LTA individuals in both stress and resting states. The study found reduced functional connectivity between the superior frontal gyrus and anterior cingulate cortex in HTA individuals, indicating that their top-down control functions are compromised not only in test situations but also in general contexts (Huang et al., 2022). The inhibition of subcortical

structures like the amygdala by the prefrontal cortex is mediated through the neurotransmitter GABA (Thayer & Lane, 2009). Studies employing magnetic resonance spectroscopy (MRS) assessed the glutamate/GABA balance in HTA individuals and found that the glutamate/GABA balance in the superior sulcus was associated with changes in current test anxiety levels and could predict future test anxiety approximately 21 months later. Importantly, this relationship was observed in early childhood but not in later developmental stages, further supporting the idea that HTA is related to a failure of cortical inhibition(Zacharopoulos et al., 2022). It is the neural physiological differences between HTA and LTA individuals that may account for the distinct relationship observed between the left DLPFC and nonlinear HRV.

Moreover, LTA individuals may inherently be in a more balanced physiological state, leading to the absence of significant HRV changes under the influence of tDCS. Previous research has indicated that patients with anxiety-related disorders exhibit an imbalance in activity between the left and right DLPFC, characterized by reduced activity in the left DLPFC and increased activity in the right DLPFC (Grimm et al., 2008; Nitschke & Heller, 2005). This imbalance between the left and right DLPFC, along with overactivity in the right DLPFC, is implicated in the processing of negative emotions and the generation of anxiety (Sagliano et al., 2016). Anodal tDCS stimulation may have a ceiling effect on already active cortical regions, and in healthy individuals, the left DLPFC is relatively active, which could be a potential reason why HD-tDCS did not effectively enhance nonlinear HRV. These explanations may warrant further investigation through brain stimulation and fMRI studies to validate their hypotheses.

The primary contribution of this study lies in the discovery of the distinct role of the left DLPFC in non-linear HRV among HTA individuals. This suggests that anodal HD-tDCS targeting the left DLPFC holds promise as a candidate intervention for test anxiety. While tDCS has been widely employed in the treatment of various anxiety-related disorders, such as generalized anxiety disorder (Lin et al., 2019), social anxiety disorder (Heeren et al., 2017), and math anxiety (Sarkar et al., 2014), there has been no prior research investigating the effectiveness of tDCS in mitigating test anxiety. Future studies can build upon this foundation to further explore the impact of tDCS on cognitive and emotional tasks in HTA individuals. Another notable contribution of this study is the initial revelation of differences in non-linear HRV during resting states between HTA and LTA individuals. Previous research has typically focused on differences in HRV during evaluative situations among HTA and LTA individuals (Bian et al., 2022). However, this study demonstrates that the divergence in autonomic responses between HTA and LTA individuals is not reflected in the magnitude of HRV during resting states. Instead, it manifests as the ability of LTA individuals to autonomously recover and elevate non-linear HRV over time, thereby enhancing their overall cognitive and emotional states. Conversely, HTA individuals lack this capacity. This finding potentially signifies dynamic deficits in

neurovisceral integration in HTA individuals, which necessitates further investigation in future research.

This study still has some limitations. Firstly, the study participants were drawn from the university students, which may limit the generalizability of the research findings to other demographic groups. Future research could consider including high school students as participants. Secondly, this study exclusively investigated the left DLPFC as a stimulation site. However, the right DLPFC and ventromedial prefrontal cortex (vmPFC) also play significant roles in anxiety and the autonomic nervous system. In future studies, alternative brain stimulation techniques like cathodal tDCS or TMS could be employed to further investigate the relationship between the prefrontal cortex and HRV in HTA individuals. This approach would provide a more comprehensive understanding of the neurophysiological mechanisms underlying test anxiety. Thirdly, this study primarily focused on objective physiological indices and did not assess subjective state anxiety changes immediately before and after the stimulation. While HRV provides a direct measure of autonomic regulation, future research should incorporate self-reported measures (e.g., State-Trait Anxiety Inventory) to investigate whether the tDCS-induced physiological improvements are accompanied by changes in subjective emotional experience.

Conclusion

Applying anodal HD-tDCS to activate the left DLPFC significantly enhances the nonlinear HRV in HTA individuals, but this manipulation is ineffective for LTA individuals.

Declarations

Author contributions statement

P.L. conceived and designed the study, curated and analysed the data, conducted the investigation, and wrote the first draft of the manuscript. S.B. contributed to data curation, formal analysis, and manuscript drafting. B.Z. contributed to data collection, validation, and manuscript revision. R.Z. contributed to conceptualization, supervision, funding acquisition, and manuscript revision. All authors reviewed and approved the final manuscript.

Ethics approval

Approval was obtained from the ethics committee of the Department of Psychology at Nanjing University. The procedures used in this study adhere to the tenets of the Declaration of Helsinki.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent for publication

All participants provided informed consent for the publication of anonymized data.

Availability of data and materials

Data are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Open practices statement

Data or materials for the experiments are available upon request, and none of the experiments was preregistered.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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