Anodal High-Definition tDCS over the Left Dorsolateral Prefrontal Cortex Is Associated with Autonomic Regulation and Executive Performance

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Abstract

High-definition transcranial direct current stimulation (HD-tDCS) is a non-invasive neuromodulation technique increasingly investigated for its association with executive function performance. Emerging evidence indicates that prefrontal stimulation may also be associated with changes in autonomic regulation, as indexed by heart rate variability (HRV)—a physiological marker linked to adaptive cognitive control and emotional regulation. Within the *Neurovisceral Integration Model*, prefrontal cortical activity supports dynamic interactions between cognitive and autonomic systems, providing a theoretical framework for examining concurrent variation in HRV and executive performance during left dorsolateral prefrontal cortex (DLPFC) stimulation. Here, we examined the effects of anodal HD-tDCS over the left DLPFC on executive performance and autonomic activity in healthy adults using a randomised, single-blind, crossover design. Participants completed a working memory (N-Back) and an inhibitory control (Stroop) task before and during either anodal or sham HD-tDCS (2 mA, 20 min), while electrocardiogram recordings were collected to derive high-frequency HRV (HF-HRV). Self-reports of negative/positive affect were also collected pre- and during-tDCS. Anodal HD-tDCS was associated with a greater increase in N-Back accuracy relative to sham stimulation. In the Stroop task, accuracy increased during stimulation, whereas reaction time reductions were observed across both stimulation conditions; the reduction in reaction time was greater under anodal than sham stimulation, although the observed pattern should be interpreted cautiously given baseline differences and possible practice-related effects. Anodal stimulation was further associated with greater increases in HF-HRV from baseline compared to sham. Significant associations between HF-HRV and executive performance emerged under anodal stimulation but not under sham. No significant changes were observed in self-reported affect. These findings are consistent with the co-occurrence of cognitive and autonomic changes during anodal stimulation of the left DLPFC, together with an observed association between HF-HRV and selected aspects of executive performance.

Keywords: HD-tDCS; left Dorsolateral Prefrontal Cortex; Heart Rate Variability; Executive Functions; Stroop Task; N-Back Task.

Introduction

Executive function (EF) refers to a set of higher-order, top-down cognitive processes—including working memory (WM), inhibitory control (IC), and cognitive flexibility/task switching—that are essential for flexible, goal-directed behaviour across the lifespan (Cristofori et al., 2019; Ferguson et al., 2021; Miyake et al., 2000). Given their fundamental role in everyday functioning, interventions designed to enhance EF have the potential to yield substantial cognitive and functional benefits across the lifespan (Frau et al., 2022; Sandberg et al., 2014).

In recent years, there has been growing interest in the use of transcranial direct current stimulation (tDCS) as a non-invasive neuromodulation technique to modulate cortical excitability, particularly within the prefrontal cortex (Imburgio & Orr, 2018; Ke et al., 2019; Sallard et al., 2018). tDCS delivers a low-intensity direct current (typically 0.5–2 mA) to targeted cortical areas via electrodes placed on the scalp (Nitsche et al., 2008; Nitsche & Paulus, 2000). This stimulation induces polarity-dependent changes in neuronal excitability, with anodal stimulation generally depolarising neuronal membranes and increasing cortical excitability, whereas cathodal stimulation tends to hyperpolarise neurons, reducing excitability. These effects are thought to involve modulation of N-methyl-D-aspartate (NMDA) receptor activity, ultimately influencing synaptic plasticity (Nitsche & Paulus, 2000; Nitsche et al., 2003a,b). Overall, tDCS is considered a safe, well-tolerated, and versatile method for investigating and modulating brain function in both healthy and clinical populations.

Building upon conventional tDCS, high-definition tDCS (HD-tDCS) employs arrays of

smaller ring-shaped electrodes rather than conventional sponge electrodes, providing greater spatial focality and more precise targeting of cortical regions, thereby improving the specificity and reliability of stimulation effects (Kuo et al., 2013; Villamar et al., 2013). HD-tDCS has demonstrated efficacy in enhancing EF in healthy adults—particularly working memory, cognitive control, and inhibitory processes (Gbadeyan et al., 2016; León-Correa et al., 2026a; Nikolin et al., 2017). Accordingly, the present study focused on working memory and inhibitory control, two executive domains consistently linked to left DLPFC functioning (Miller & Cohen, 2001; Gbadeyan et al., 2016; León-Correa et al., 2026b; Nikolin et al., 2017).

Although executive functions rely on distributed bilateral frontoparietal networks, the left DLPFC represents an important node supporting cognitive flexibility, working-memory and cognitive-control processes relevant to the N-Back and Stroop tasks, including interference resolution (Barbey et al., 2013; Huang et al., 2020; León-Correa et al., 2026b; Lu et al., 2021; Nee et al., 2013). Previous studies have also associated anodal HD-tDCS over the left DLPFC with changes in executive task performance, including working memory, divided attention, and inhibitory control (Lu et al., 2021; León-Correa et al., 2026a; Nikolin et al., 2017).

Beyond its role in executive processing, the DLPFC is also considered a cortical component of the central autonomic network (CAN), contributing to top-down autonomic regulation through its interactions with cortical and subcortical structures (Benarroch, 1993, 1997; Breit et al., 2018; Schmaußer et al., 2024). This dual involvement provided the rationale for examining cognitive and autonomic outcomes within the same experimental framework.

The relationship between executive functioning and autonomic regulation is formalised by the Neurovisceral Integration Model (NIM) proposed by Thayer and Lane (2000), which posits that cognitive control and cardiac vagal regulation rely on interconnected neural systems. According to this model, bidirectional communication between the brain and the heart is mediated by the CAN, comprising cortical and subcortical regions—including the prefrontal cortex, hypothalamus, and brainstem—that regulate autonomic responses through the vagus

nerve (Benarroch, 1993, 1997; Breit et al., 2018; Thayer & Lane, 2009). Within this framework, the left DLPFC is thought to influence autonomic function through its connections with subcortical autonomic centres (Schmaußer et al., 2022).

Heart rate variability (HRV), particularly high-frequency HRV (HF-HRV), is widely recognised as a non-invasive marker of parasympathetic activity and an indicator of central autonomic regulation (Shaffer & Ginsberg, 2017; Thayer & Lane, 2009). HRV reflects beat-to-beat variation in cardiac activity and is considered an important physiological index of adaptive self-regulation (Mohammadpoor Faskhodi et al., 2023; Shaffer et al., 2014). It also plays a significant role in supporting behavioural flexibility (Thayer et al., 2009, 2012). Higher HRV has consistently been associated with superior executive functioning (Forte et al., 2019; Nicolini et al., 2024), enhanced emotional regulation (Appelhans & Luecken, 2006), greater resilience to stress (Kim et al., 2018; Spangler et al., 2018), and stronger functional connectivity within prefrontal–limbic networks involved in emotion regulation (Mather & Thayer, 2018; Thayer et al., 2012). High levels of parasympathetic activity, as indexed by HRV, may also contribute to slowing cognitive decline across the lifespan (Nicolini et al., 2024). More broadly, greater parasympathetic modulation has been linked to adaptive cognitive and emotional responding (Thayer & Friedman, 2004; Thayer et al., 2009). Collectively, these findings indicate that executive performance and autonomic regulation are closely interconnected rather than independent processes.

Consistent with the NIM, growing evidence suggests that prefrontal tDCS may influence autonomic regulation by modulating HRV. Ko et al. (2024) recently provided meta-analytic evidence showing that active tDCS enhances HRV compared with sham across multiple experimental conditions. Similarly, Carnevali et al. (2020) demonstrated that anodal stimulation of the left DLPFC increased HRV both at rest and during psychosocial stress in healthy young adults. Brunoni et al. (2013) reported enhanced vagal activation, reflected by increased HF (0.15–0.4 Hz) power, during an emotional picture-viewing task following anodal stimulation of

the left DLPFC. Likewise, Nikolin et al. (2017) reported significantly higher HF-HRV during a working-memory task under bifrontal tDCS, consistent with an effect of prefrontal stimulation on cardiac parasympathetic activity. More broadly, these findings link left DLPFC stimulation to both executive and autonomic outcomes within the NIM framework.

Beyond its role in executive functioning and autonomic regulation, the DLPFC also contributes to emotional regulation, including mood and emotional reactivity (Habel et al., 2005; Kredlow et al., 2022; Zhang et al., 2022). Both single and repeated sessions of anodal tDCS over the DLPFC have been shown to improve emotional regulation by enhancing cognitive control over emotional responses and reducing reactivity to negative stimuli (Clarke et al., 2020; Molavi et al., 2020). These effects have been demonstrated using both subjective measures, such as the Positive and Negative Affect Schedule (PANAS; Wiegand et al., 2019), and physiological indices including HR and skin conductance (Carnevali et al., 2020; Feeser et al., 2014), further supporting the close interaction between prefrontal stimulation, emotional regulation, and autonomic function.

However, although conventional tDCS has shown promising effects on cardiac vagal control and emotional processing, evidence regarding its impact on executive functioning and the interaction between cognitive and autonomic regulation remains limited and inconsistent. Moreover, the potential of HD-tDCS, which provides greater focality and cortical specificity than conventional tDCS, has been scarcely investigated in this context. Therefore, the present study simultaneously examined executive performance and HF-HRV during anodal HD-tDCS over the left DLPFC to investigate whether focal stimulation of this region influences both cognitive and physiological outcomes within the same experimental framework.

The Current Study

The present study sought to clarify these relationships by examining the associations between HD-tDCS over the left DLPFC, self-reported affect, cardiac HF-HRV, and key executive

functions. Specifically, we examined the effects of anodal compared with sham HD-tDCS over the left DLPFC on positive and negative affect, cardiac HF-HRV, and executive functioning (EF)—specifically working memory (WM) and inhibitory control (IC)—in a sample of healthy adults. Although cognitive flexibility is recognised as a core component of executive functioning, it was not directly assessed in the present study. Instead, we focused on working memory and inhibitory control, as these domains are consistently linked to left DLPFC function and can be reliably evaluated during HD-tDCS using validated paradigms (N-Back and Stroop tasks).

We employed a randomised, single-blind, crossover design, in which each participant underwent both sham and anodal stimulation in separate sessions. Positive and negative affect were measured via the PANAS, and electrocardiogram (ECG) recordings were used to derive HF-HRV, as an index of cardiac vagal tone. WM capacity was assessed using the N-Back task, while IC was evaluated using the Stroop task. The N-Back task included 0-, 1-, and 2-back conditions to examine performance across increasing levels of working-memory demand. These workload levels were included to determine whether any stimulation-related effects differed according to task demand; however, no specific a priori hypothesis was formulated regarding workload-dependent effects.

We hypothesised that anodal HD-tDCS, compared to sham stimulation over the left DLPFC, would be associated with greater pre- to during-tDCS improvements in executive-task performance, as reflected in behavioural indices of speed and/or accuracy, and with a greater increase in HF-HRV. Changes in affective state were explored as a secondary outcome. Specifically, we expected that left DLPFC stimulation would be associated with greater improvements in cognitive-control performance and potentially with changes in affective responding (Clarke et al., 2020; Feeser et al., 2014), particularly under cognitively demanding conditions such as those induced by the Stroop task (Kuehne et al., 2019; Wiegand et al., 2019).

In line with the NIM (Thayer & Lane, 2000), we further hypothesised that anodal HD-tDCS would be associated with a greater pre- to during-tDCS increase in HF-HRV than sham stimulation, consistent with greater parasympathetic activity. Importantly, we sought to determine whether changes in HF-HRV were associated with concurrent changes in executive performance within each stimulation condition.

Methods

Participants

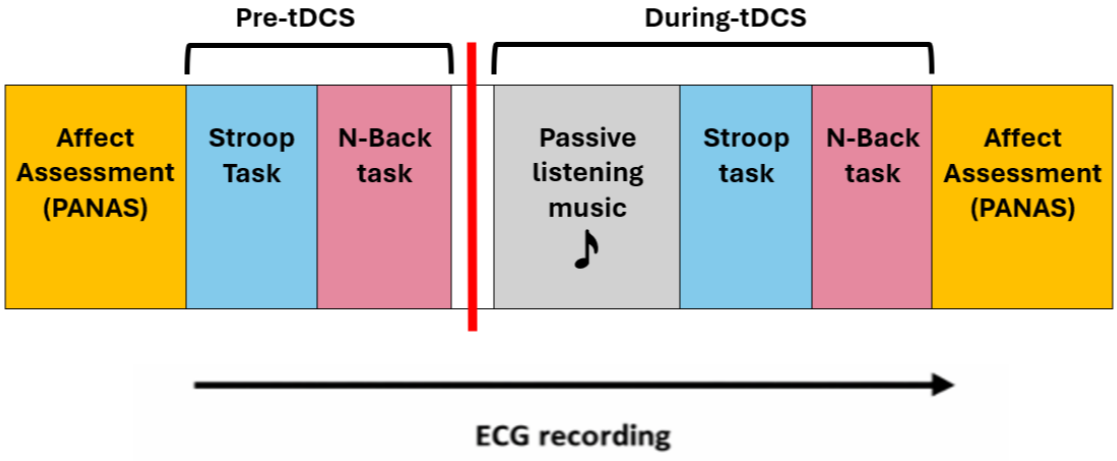
An a priori power analysis was conducted using G*Power 3.0.10 (Faul et al., 2007) for a repeated-measures design. Assuming a large effect size ($f^2 = 0.40$), $\alpha = .05$, and power = .80, the minimum required sample size was estimated at 33 participants. This estimate was informed by previous reports of tDCS-related effects across cognitive and motor domains (Jacobson et al., 2012). To account for potential attrition, a total of 38 healthy adults were recruited. The final sample comprised 36 healthy adults (mean age = 46.75 years, SD = 21.74; range = 19–75 years). To further characterise the broad age distribution, 15 participants were aged 19–35 years, 8 were aged 36–55 years, and 13 were aged 56–75 years. All participants but three were right-handed as ascertained by means of the Edinburgh Handedness Inventory (Oldfield, 1971). They were all fluent in English and reported no history of neurological or psychiatric disorders, substance or alcohol abuse, cardiovascular disease, or other severe medical conditions. Participants were not taking medication known to affect cortical excitability or autonomic function. Participation was compensated with a £10 shopping voucher, and undergraduate students enrolled at [name of the institution] additionally received SONA course credit. All participants provided written informed consent. The study was approved by the [name of the institution] Research Ethics Committee [ID]. The experiment was not preregistered.

General procedure

The study timeline is illustrated in Figure 1. On two separate days, participants completed laboratory sessions using a single-blind, randomised crossover design. In each session, they received either anodal or sham HD-tDCS in counterbalanced order, with at least 48 hours between sessions to minimise carryover effects. Stimulation order (anodal vs. sham) was counterbalanced across participants in a randomised crossover design. The order of cognitive task administration (Stroop and N-Back) was also counterbalanced within each stimulation session to minimise order effects. At the beginning of each session, participants completed a safety screening questionnaire (Rossi et al., 2011), followed by the Edinburgh Handedness Inventory (Oldfield, 1971) and a demographic questionnaire. Each session consisted of two testing phases: pre-tDCS (baseline) and during-tDCS. ECG data were recorded continuously throughout the experiment to compute frequency-domain HRV, focusing on high-frequency (HF; 0.15–0.4 Hz) power. During the pre-tDCS phase, participants first completed the Positive and Negative Affect Schedule (PANAS; Watson, 1988) to assess affective state and then performed the Stroop and N-Back tasks (order counterbalanced across participants) using Inquisit 6 (Millisecond software, 2023), following standardised instructions and a short practice. Following completion of the baseline tasks, HD-tDCS was initiated at the beginning of a 10-minute resting interval during which participants listened passively to Beethoven's Symphony No. 7 in A major, Op. 92, following Baumert et al. (2020), to minimise additional cognitive load during stimulation. Stimulation then continued for a further 10 minutes while participants repeated the cognitive tasks (during-tDCS phase). All participants completed the cognitive tasks within the second 10-minute period, following an initial 10-minute passive stimulation phase during which they listened to music; therefore, stimulation did not exceed the planned total duration of 20 minutes. In the anodal condition, HD-tDCS was applied continuously for 20 minutes; in the sham condition, current was delivered only briefly at the start to reproduce the initial sensations of active stimulation (see Methods below). At the end of each session, participants again completed the PANAS (Wiegand et al., 2019) and indicated whether they

believed they had received sham or anodal stimulation (Stanković et al., 2021; see Supplemental Material).

Figure 1: Timeline of the HD-tDCS Experimental Procedure, Including Affective, Physiological, and Cognitive Assessments.



Note. *PANAS* = Positive and Negative Affect Schedule; ECG = electrocardiogram signals used to compute high-frequency heart rate variability (HF-HRV). *Pre-tDCS:* Following completion of the PANAS, participants performed the Stroop and N-Back tasks (task order counterbalanced) prior to the onset of HD-tDCS stimulation. *Passive music listening (stimulation onset):* HD-tDCS (anodal or sham) was initiated at the beginning of a 10-minute passive music-listening interval, during which participants listened to music to minimise cognitive load. *During-tDCS:* After the initial 10-minute passive phase, stimulation continued for an additional 10 minutes while participants performed the cognitive tasks. Total stimulation duration was 20 minutes.

Positive and Negative Affect Schedule (PANAS)

We used the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), which consists of a 20-item self-report measure to assess dispositional affect before and during-tDCS

248 stimulation. It comprises two 10-item subscales: Positive Affect (PA) and Negative Affect
249 (NA). Higher scores indicate greater levels of each affective dimension. High PA reflects
250 energy, concentration, and pleasurable engagement, whereas low PA indicates sadness and
251 lethargy. High NA reflects emotions such as anger, guilt, fear, and nervousness, while low NA
252 indicates calmness. Participants rated each item on a 5-point scale from 1 ("very slightly or not
253 at all") to 5 ("extremely").
254
255 **Cognitive Tasks**
256 **Stroop task**
257 The Stroop task (Stroop, 1935) measures the ability to inhibit automatic responses by
258 requiring participants to ignore the meaning of a word and focus on naming the colour of the
259 word's ink. In this study, participants were asked to type specific keys corresponding to the
260 colour of the word displayed on the screen [i.e., D = red, F = green, J = blue, and K = yellow]
261 as quickly and accurately as possible. Each word was displayed until one of the four keys was
262 pressed. The task included a total of 84 trials [4 colours × 3 stimuli (congruent, incongruent,
263 control) × 7 repetitions]. This resulted in 28 congruent trials (word and colour match), 28
264 incongruent trials (word and colour do not match), and 28 control trials (coloured rectangles),
265 randomly presented (Parkin et al., 2017). Prior to the start of the task, participants were trained
266 with a short practice consisting of 12 practice trials (4 for each trial type). During the
267 experiment, if the response was correct, the subsequent trial started immediately. Accuracy was
268 calculated as the percentage of correct responses (% accuracy = total correct responses / number
269 of trials), with correct responses coded as 1 and incorrect responses coded as 0. RTs were
270 recorded by measuring the time lapse between the presentation of the stimulus and the
271 participant's response on the keyboard. For our analyses, we calculated the mean latency of
272 congruent or incongruent trials (in msec) to assess response times (RTs) in each condition (sham

vs. anodal, pre-tDCS and during-tDCS). Data from the practice trials were not included in the accuracy and RTs performance counts.

N-Back

The N-Back task (Kirchner, 1958) was used to assess WM capacity by asking participants to complete three N-levels of difficulty. For N=0 trials, participants had to indicate any letter that matched a pre-specified letter. For N=1 trials, participants have to determine if the current letter is the same as the letter that was presented immediately before it. For N=2 trials, participants need to determine if the current letter is the same as the letter presented in the two previous trials. Participants indicated their responses using two separate keys (0 and 1) on their computer keyboard.

During the experiment, the N-Back levels were randomly presented (each N-level was shown twice), with instructions displayed for 9000 milliseconds before each level. For each N-Back level, 15 consonants were presented, with a total of 90 stimuli. Each letter appeared one at a time in the centre of the computer monitor for 500 milliseconds with 33% of target letters (i.e., 1:2 ratio as in Ragland et al., 2002). To assess accuracy, we used the percentage of "hits", which indicated instances where participants correctly recognised matching stimuli in the n-back task. Performance accuracy was calculated as the percentage of correct responses (% accuracy = total correct responses / total number of trials) at each n-back level, with responses coded as 1 (correct) and 0 (incorrect). Practice trials (2 trials for each N-level) were not included in the performance measurements for RTs and accuracy. The Stroop and N-Back tasks were programmed and administered using Inquisit 6 (Millisecond Software, 2023), with task scripts customised for the specific protocol of this study.

Physiological measures and apparatus

An ECG signal was collected using a Biopac System, Inc., MP36 to assess vagal tone by

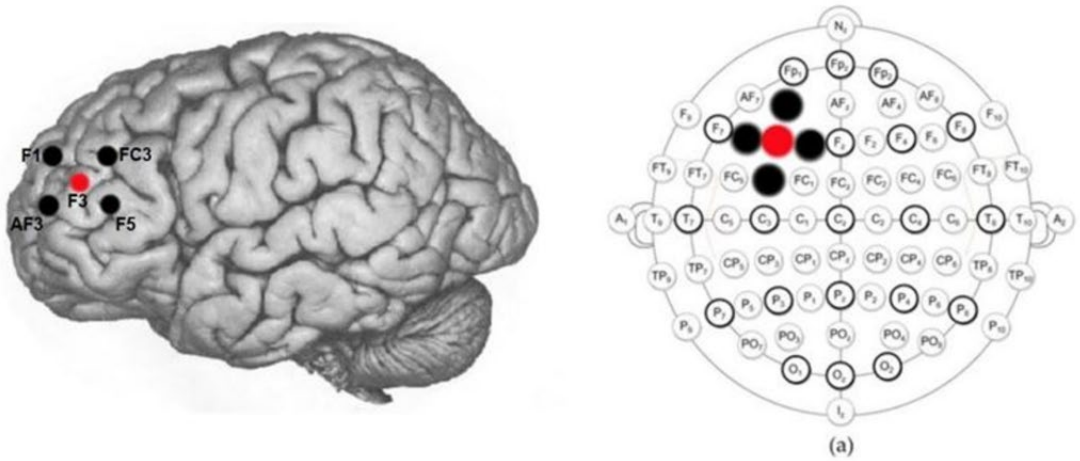
measuring HF-HRV (variation in time between each heartbeat for high power frequency) in the range of 0.15-0.4 Hz. This parameter serves as an index of the parasympathetic nervous system activity (Dantas et al., 2012). For our analyses, we considered two temporal windows: HF-HRV at pre-tDCS (baseline) and during-tDCS. Prior to each task, participants were instructed to remain still and relaxed with their eyes open for 3 minutes, a sufficient time interval for obtaining accurate measures of emotional arousal (Della Longa et. al., 2021; Munoz et al., 2015). To record ECG signals, three electrodes were positioned on the torso in a standard configuration approximating Einthoven's triangle (i.e., one electrode placed below each clavicle and one on the lower left torso/hip). The electrodes were connected to the BIOPAC acquisition system (Student Lab PRO v3.7), which applied a real-time band-pass filter of 0.5–35 Hz during signal acquisition. The recordings were interspersed with 30s breaks, and the sampling rate for data acquisition was set at 2000Hz. ECG signals were first visually inspected to remove artefacts and then imported into Kubios HRV software (Tarvainen et al., 2014) to obtain the frequency domain measure of the High-Frequency band (i.e., 0.15-0.4 Hz). From the original ECG signal, the software retrieves the interbeat (or RR) intervals and applies the smoothness prior's method to remove the low-frequencybaseline trend component. The normalised high-frequency HF-HRV units were acquired through frequency domain estimation employing power spectrum density. This estimation method involved Welch's periodogram method, which leverages the fast Fourier transformation (Welch, 1967).

High-definition transcranial direct current stimulation protocol

HD-tDCS was delivered using a DC-stimulator PLUS (Neuroconn DC stimulator PLUS-Rogue Resolutions) over the left DLPFC. The protocol utilised a "4×1 ring electrode configuration" (Villamar et al., 2013), with a 12 mm anode placed on location F3 and four -0.5 mA cathodes placed on AF3, F1, F5, and FC3 (Guo et al., 2018; Zhou et al., 2023) (see Figure 2). The targeted area was localised with an EEG cap laid out according to the International 10-

20 EEG System. An elastic tDCS cap was used to ensure the electrodes remained firmly positioned during stimulation. A conductive gel was placed over the scalp under the hair to ensure connectivity.

Figure 2: 4×1 ring montage on the left DLPFC: HD-tDCS protocol



Note. Electrode placement for the HD-tDCS protocol using a 4×1 ring configuration. According to the 10–20 International EEG system, electrodes were positioned over the left dorsolateral prefrontal cortex (DLPFC): black sites indicate the cathodes (AF3, F1, F5, and FC3), and the red site indicates the anode (F3).

For the anodal condition, HD-tDCS was applied at 2 mA for 20 min, with a 30 s ramp-up and a 30 s ramp-down. During sham stimulation, the current was increased to 2 mA over 30 s, maintained for 30 s to elicit paraesthesia and preserve blinding, and then decreased over the following 30 s (Palm et al., 2013; Zawertailo et al., 2022). In both conditions, the total stimulation duration was fixed at 20 min. During the first 10 min, participants remained seated while listening to relaxing music. During the final 10 min, while stimulation was still active, participants completed the Stroop and N-Back tasks; thus, all cognitive performance measures were obtained during online HD-tDCS. This protocol was designed to examine stimulation-

related changes during active left DLPFC HD-tDCS while executive networks were concurrently engaged, consistent with evidence that tDCS responses are state-dependent and may vary when stimulation is paired with concurrent cognitive task performance (Martin et al., 2013; Fertonani & Miniussi, 2017; Polanía et al., 2018). An interval of at least 48 hours between the two sessions was used to minimise the potential for cumulative or carryover effects, consistent with washout intervals employed in previous tDCS studies (Grandperrin, 2020; Shekhawat & Vanneste, 2018). This interval exceeds the duration of the behavioural and physiological after-effects typically reported following a single session of tDCS (Nitsche & Paulus, 2001; Nitsche et al., 2003b). Although HD-tDCS provides greater spatial focality than conventional tDCS, there is currently no evidence that a single HD-tDCS session produces after-effects extending beyond this interval (Kuo et al., 2013). The study was conducted in accordance with the safety and ethical guidelines produced by Rossi et al. (2009, 2021).

Data handling

All statistical analyses were performed using IBM SPSS Statistics 29 (SPSS Inc., Chicago, IL, USA). Prior to analysis, the normality of dependent variables across tasks (Stroop, N-Back), stimulation conditions (anodal, sham), and time points (pre-tDCS, during-tDCS) was assessed using the Shapiro–Wilk test. Reaction times (RTs) data remained non-normally distributed despite logarithmic (Log10) and square root transformations. Given the within-subject design and sample size (N = 36), parametric analyses were considered robust to moderate deviations from normality (Ghasemi & Zahediasl, 2012). Outliers were inspected via boxplots and defined as values exceeding ±3 SD from the condition mean. Two participants were excluded on this basis (Baumert et al., 2020) from subsequent analyses. Mean accuracy and RTs were calculated for each task, averaging across congruency levels (congruent, incongruent) in the Stroop task and across workload levels (0-, 1-, and 2-back) in the N-Back task. Mean HF-HRV values were

computed separately for pre-tDCS and during-tDCS phases. Homogeneity of variance and sphericity assumptions were verified prior to ANOVA testing.

To examine potential systematic baseline differences between sessions that might be consistent with carryover effects, pre-tDCS baseline measures were compared between the sham and anodal sessions using two-tailed paired-samples t-tests. Bonferroni correction was applied across the six physiological and cognitive baseline comparisons. Baseline values are presented descriptively in Table 1, whereas the corresponding paired comparisons, including unadjusted and Bonferroni-adjusted p values, are reported in Supplementary Table S1.

All analyses followed a predefined statistical pipeline designed to evaluate stimulation-related changes in affective, autonomic, and cognitive responses. First, a 3-way repeated-measures ANOVA was conducted on PANAS scores, with Time (pre- vs. during-tDCS), Stimulation (sham vs. anodal), and Valence (positive vs. negative) as within-subject factors. HF-HRV data were then analysed using a similar 3-way repeated-measures ANOVA with Time (pre- vs. during-tDCS), Stimulation (sham vs. anodal), and Task (Stroop vs. N-Back) as factors, allowing us to quantify autonomic modulation across cognitive contexts. Cognitive performance was examined through two separate 3-way repeated-measures ANOVAs reflecting the structure of each task: the Stroop analysis included Time (pre- vs. during-tDCS), Stimulation (sham vs. anodal), and Congruency (congruent vs. incongruent), whereas the N-Back analysis included Time (pre- vs. during-tDCS), Stimulation (sham vs. anodal), and Workload (0-, 1-, and 2-back). All data are reported as means (M), with standard deviations (SD) for baseline measures and as means (M) with standard errors of the mean (SEM) for repeated task measures across Time, Stimulation, and task-specific conditions. Graphical distributions are displayed using boxplots showing medians, interquartile ranges, and individual participant values. Statistical significance was set at $p < .05$, and effect sizes were estimated

using partial eta squared (η^2_p). Significant interactions were explored using Tukey-adjusted post hoc comparisons.

To examine associations between autonomic activity and executive performance, change scores (Δ = during-tDCS − pre-tDCS) were calculated for each behavioural outcome. Negative Δ values indicated reduced RTs, whereas positive Δ values indicated increased accuracy. Pearson correlations between HF-HRV and behavioural change scores were conducted separately for the sham and anodal conditions, with Bonferroni correction applied separately within each task domain.

Supplementary analyses further examined the Stroop RT findings. Sham and anodal ΔRT scores were compared using a paired-samples t-test (Supplementary Table S2), and Pearson correlations between baseline RTs and the corresponding ΔRT scores were calculated within each stimulation condition (Supplementary Table S3). Bonferroni correction was applied across the three supplementary Stroop RT analyses reported in Supplementary Tables S2 and S3.

Exploratory linear regression analyses were also conducted to examine whether age predicted the differential stimulation effect (ΔΔ = anodal Δ − sham Δ) for each physiological and behavioural outcome (Supplementary Table S4).

The effectiveness of participant blinding was evaluated at the end of each experimental session using a forced-choice procedure. Binomial tests were used to compare the proportion of correct guesses against chance level (50%). To examine whether perceived stimulation condition influenced behavioural performance, participants who correctly and incorrectly identified the stimulation condition were compared using speed–accuracy trade-off indices (Supplementary Material).

Results

Descriptive statistics

Baseline pre-tDCS measures, including positive and negative affect (PANAS), HF-HRV, and cognitive performance (RTs and accuracy) on the Stroop and N-Back tasks, are presented for both sham and anodal sessions in Table 1. For the six physiological and cognitive baseline comparisons, Bonferroni correction was applied to account for multiple testing. No between-session differences remained statistically significant after correction (all adjusted $p \geq .114$). Although HF-HRV during the N-Back task differed at the unadjusted level ($p = .019$), this comparison was no longer significant after correction (adjusted $p = .114$). All other affective, physiological, and behavioural measures were comparable between sessions.

Table 1: Baseline affective, physiological, and cognitive measures in the sham and anodal HD-tDCS sessions

Variable	Sham Mean ± SD	Anodal Mean ± SD	*p*	Bonferroni-adjusted *p*	Cohen's *d*
Affect					
PANAS Positive	29.560 ± 6.407	28.000 ± 6.829	.184	-	0.226
PANAS Negative	11.810 ± 2.539	11.220 ± 1.822	.128	-	0.260
Cardiac arousal					
HF-HRV	50.830 ± 9.475	51.478 ± 9.156	.102	.612	0.280

Variable	Sham Mean ± SD	Anodal Mean ± SD	*p*	Bonferroni-adjusted *p*	Cohen's *d*
Stroop					
HF-HRV N-Back	51.137 ± 9.169	52.815 ± 8.987	.019	.114	0.411
Stroop task					
RTs	1076.708 ± 166.652	1084.181 ± 152.354	.726	1.000	0.059
Accuracy (%)	80.778 ± 5.962	81.306 ± 6.610	.649	1.000	0.077
N-Back task					
RTs	1492.972 ± 122.324	1455.269 ± 135.319	.146	.876	0.248
Accuracy (%)	58.982 ± 9.148	61.204 ± 9.125	.081	.486	0.299

Note. Values are presented as mean ± SD. HF-HRV = high-frequency heart rate variability; baseline HF-HRV represents values obtained during the pre-tDCS phase and is reported separately for the Stroop and N-Back task periods. Stroop reaction time represents the mean across congruent and incongruent trials, and accuracy represents the percentage of correct responses across Stroop trials. N-Back reaction time represents the mean across the 0-, 1-, and 2-back conditions, and accuracy represents the percentage of correct responses averaged across the three workload conditions. Sham and anodal pre-tDCS values were compared using two-tailed paired-samples t-tests. Bonferroni-adjusted p values were calculated across the six physiological and cognitive baseline comparisons. Cohen's d was calculated using the standard

deviation of the paired differences. SD = standard deviation; RT = reaction time.

PANAS Outcome

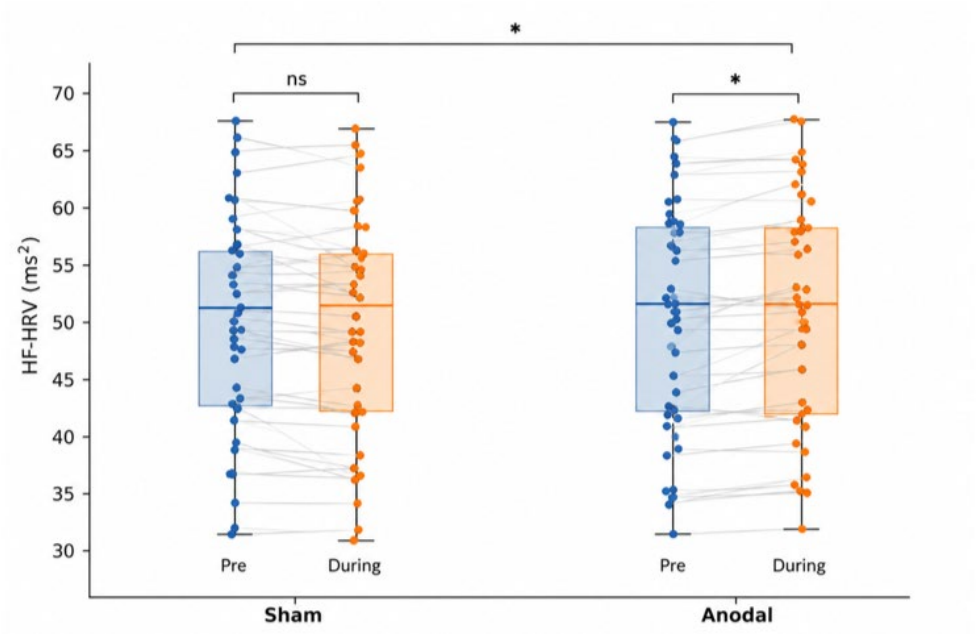
A significant Time × Valence interaction was observed [$F(1, 35) = 4.182$, $p = .048$, $\eta^2_p = .107$]. Post hoc comparisons showed that positive affect scores were significantly higher than negative affect scores at both pre-tDCS (positive: $M = 28.780 \pm 0.940$; negative: $M = 11.510 \pm 0.320$; $p < .001$) and during-tDCS (positive: $M = 29.250 \pm 0.920$; negative: $M = 11.360 \pm 0.310$; $p < .001$). However, no significant change over time was observed for either positive affect (pre-tDCS vs. during-tDCS: $p = .147$) or negative affect (pre-tDCS vs. during-tDCS: $p = .894$). Thus, the significant interaction was not associated with a change in affect overt time but rather reflected the consistently higher positive than negative affect ratings across both assessment time points. For completeness, a significant main effect of Valence was also observed [$F(1, 35) = 392.201$, $p < .001$, $\eta^2_p = .918$], with overall higher positive than negative affect ratings ($M = 29.010 \pm 0.930$ vs. $M = 11.440 \pm 0.300$). No other main effects or interactions reached significance (all $Fs < 2.631$, all $ps > .114$). Overall, these findings were not consistent with a significant association between HD-tDCS and changes in participants' self-reported affective state, as affect ratings remained stable across stimulation conditions.

High-Frequency Heart Rate Variability (HF-HRV)

The 3-way ANOVA on HF-HRV levels revealed a significant Time × Stimulation interaction [$F(1, 35) = 4.238$, $p = .047$, $\eta^2_p = .108$], showing that the change in HF-HRV from pre-tDCS to during-tDCS differed between stimulation conditions (Fig. 3). Post hoc comparisons showed a significant increase in HF-HRV during the anodal condition, from 54.461 ± 1.559 at pre-tDCS to 54.983 ± 1.546 during-tDCS ($p < .001$), whereas no significant change was observed during the sham condition (pre-tDCS: 53.579 ± 1.657; during-tDCS: 53.776 ± 1.620; $p = .310$). HF-HRV was also significantly higher in the

anodal than in the sham condition at both pre-tDCS (54.461 ± 1.559 vs. 53.579 ± 1.657, $p < .001$) and during-tDCS (54.983 ± 1.546 vs. 53.776 ± 1.620, $p < .001$). Thus, the interaction was primarily characterised by a significant within-session increase in HF-HRV during anodal stimulation that was not observed during sham stimulation. For completeness, significant main effects of Time, Task, and Stimulation were also observed. HF-HRV was higher during-tDCS than at pre-tDCS [$F(1, 35) = 12.282$, $p < .001$, $\eta^2_p = .260$; 54.379 ± 1.569 vs. 54.020 ± 1.597]. HF-HRV was also higher during the N-Back task than during the Stroop task [$F(1, 35) = 6.579$, $p = .015$, $\eta^2_p = 0.158$; 54.946 ± 1.675 vs. 53.454 ± 1.540], and higher overall during anodal than sham stimulation [$F(1, 35) = 6.877$, $p = .013$, $\eta^2_p = 0.164$; 54.722 ± 1.551 vs. 53.677 ± 1.637]. However, given the significant Time × Stimulation interaction, these main effects should be interpreted in the context of the differential change in HF-HRV across stimulation conditions. No other main effects or interactions reached significance (all $Fs < 1.304$, all $ps > .261$).

Figure 3: High-Frequency Heart Rate Variability (HF-HRV) during task performance under sham and anodal HD-tDCS over the left dorsolateral prefrontal cortex.



Note. HF-HRV = high-frequency heart rate variability. Values represent HF-HRV averaged across the Stroop and N-Back tasks. Boxplots show the median (horizontal line), interquartile range (box), and whiskers extending to values within 1.5 × the interquartile range. Each dot represents an individual participant, and grey lines connect paired observations across time points. Pre-tDCS = baseline, recorded without active HD-tDCS; during-tDCS = HF-HRV recorded during task performance under sham or anodal HD-tDCS. $p < .05$; ns = non-significant.

Overall, these findings indicate that anodal HD-tDCS may be associated with a greater within-session increase in parasympathetic activity compared with sham stimulation, independent of task.

Cognitive Tasks

Stroop task

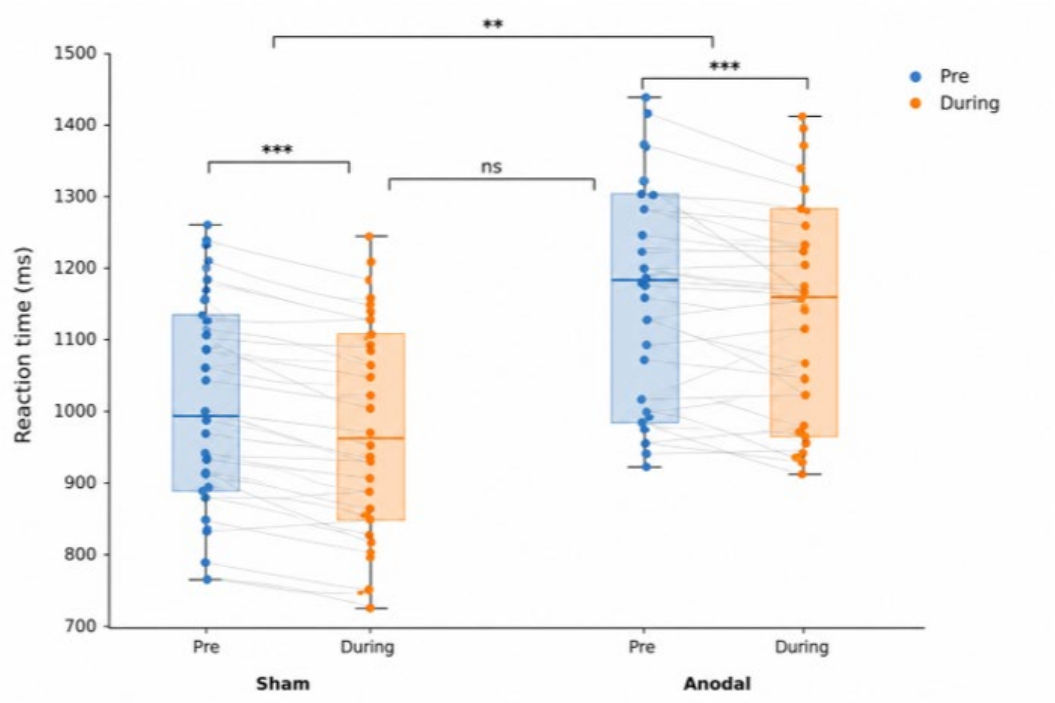
Reaction times

The 3-way ANOVA on mean RTs in the Stroop task revealed a significant Time × Stimulation interaction [$F(1, 35) = 9.353$, $p = .004$, $\eta^2_p = .211$], indicating that changes in performance from pre-tDCS to during-tDCS differed between sham and anodal conditions (see

Fig. 4). Post hoc comparisons showed that in the sham condition, RTs significantly decreased from pre-tDCS (1076.708 ± 27.775 ms) to during-tDCS (1066.958 ± 27.716 ms; $p < .001$). Similarly, in the anodal condition, RTs decreased from pre-tDCS (1084.181 ± 25.392 ms) to during-tDCS (1065.278 ± 25.705 ms; $p < .001$). When comparing stimulation conditions, RTs at pre-tDCS were significantly slower under anodal (1084.181 ± 25.392 ms) than sham stimulation (1076.708 ± 27.775 ms; $p = .006$). By contrast, during-tDCS, no significant difference emerged between sham (1066.958 ± 27.716 ms) and anodal stimulation (1065.278 ± 25.705 ms; $p = .857$).

For completeness, a significant main effect of Time was observed [$F(1, 35) = 56.325$, $p < .001$, $\eta^2_p = .617$]. Overall, lower RTs were observed during tDCS (1066.118 ± 24.454 ms) than at pre-tDCS (1080.444 ± 24.417 ms; $p < .001$). The main effect of Congruency was also significant [$F(1, 35) = 61.934$, $p < .001$, $\eta^2_p = . 0.639$], with lower RTs for congruent trials (1003.132 ± 23.180 ms) than for incongruent trials (1143.431 ± 28.530 ms; $p < .001$). No other main effects or interactions reached significance (all $Fs < 1.545$, all $ps > .222$).

Figure 4: Stroop reaction times at pre- and during-tDCS under sham and anodal HD-tDCS over the left dorsolateral prefrontal cortex.



Note. RT = reaction time. Reaction times are presented as individual participant values, with paired observations connected across time points. Boxplots show the median (horizontal line), interquartile range (box), and whiskers extending to values within 1.5 × the interquartile range. Each dot represents an individual participant, and grey lines connect paired observations across time points within each stimulation condition. Pre-tDCS = baseline; during-tDCS = task performance during sham or anodal HD-tDCS. **p < .01; ***p < .001; ns = non-significant.

Taken together, RTs decreased over time in both conditions, with anodal stimulation being associated with a greater within-session reduction than sham. To further assess whether the observed reaction time pattern reflected baseline-dependent practice effects, supplementary analyses comparing change scores (ΔRTs) between stimulation conditions and examining the association between baseline reaction times and ΔRTs were performed (Supplementary Material). These analyses did not provide evidence that the differential change in RTs between stimulation conditions was explained by baseline RT differences.

Accuracy

Mean accuracy (%) as a function of Time, Stimulation, and Congruency is presented in Table 2. The 3-way ANOVA on mean accuracy in the Stroop task revealed a significant Time × Congruency interaction [$F(1, 35) = 7.916$, $p = .008$, $\eta^2_p = 0.184$], indicating that differences in accuracy between congruent and incongruent trials varied over time, regardless of stimulation condition. Post hoc comparisons showed that for congruent trials, accuracy increased from pre-tDCS (83.500% ± 0.953) to during-tDCS (88.042% ± 0.836; $p < .001$). For incongruent trials, accuracy increased from pre-tDCS (78.583% ± 1.025) to during-tDCS (81.778% ± 0.990; $p < .001$). When comparing congruency conditions, participants were significantly less accurate on incongruent than on congruent trials both at pre-tDCS ($p < .001$) and during-tDCS ($p < .001$). For completeness, a significant main effect of Time was observed [$F(1, 35) = 244.241$, $p < .001$, $\eta^2_p = .875$]. Higher accuracy was observed during-tDCS (84.910 ± 0.832) than at pre-tDCS (81.042 ± 0.878; $p < .001$). The main effect of Congruency was also significant [$F(1, 35) = 47.613$, $p < .001$, $\eta^2_p = .576$], with higher accuracy for congruent trials (85.771 ± 0.881) than for incongruent trials (80.181 ± 0.992; $p < .001$). The Time × Stimulation interaction approached significance [$F(1, 35) = 4.085$, $p = .051$, $\eta^2_p = .105$]. Post hoc comparisons revealed that at pre-tDCS, accuracy did not differ between sham (80.778% ± 0.994) and anodal (81.306% ± 1.102; $p = .300$) stimulation. During-tDCS, however, accuracy was higher under anodal (85.597% ± 1.053) than sham stimulation (84.222% ± 0.974; $p < .001$). Within each stimulation condition, accuracy increased significantly from pre-tDCS to during-tDCS (sham: $p < .001$; anodal: $p < .001$). No other main effects or interactions reached significance (all $F < 1.726$, all $p > .197$).

Table 2. Mean accuracy (%) ± standard error of the mean (SEM) as a function of Time, Stimulation, and Congruency.

Time	Stimulation	Congruent (%)	Incongruent (%)
Pre	Sham	83.420 ± 1.090	78.140 ± 1.270
Pre	Anodal	83.580 ± 1.250	79.030 ± 1.260
During	Sham	87.280 ± 1.040	81.170 ± 1.220
During	Anodal	88.810 ± 1.110	82.390 ± 1.200

Note. Values represent mean accuracy percentages (± SEM) for the Stroop task. Pre-tDCS = baseline (Stroop task performed without HD-tDCS stimulation); during-tDCS = during stimulation (tasks performed under sham or anodal HD-tDCS). Congruent trials refer to trials in which word meaning and ink colour matched, whereas incongruent trials refer to trials in which word meaning and ink colour did not match. SEM = standard error of the mean.

Taken together, accuracy increased significantly from pre-tDCS to during-tDCS across stimulation conditions. Anodal stimulation was associated with higher accuracy during tDCS than sham stimulation; however, this finding should be interpreted cautiously because the Time × Stimulation interaction only approached significance. Accuracy was consistently higher for congruent than incongruent trials, with a greater increase over time for congruent than incongruent trials.

N-Back Task

Reaction times

Means for reaction times (ms) by Time, Stimulation, and Workload are presented in Table 3. The 3-way ANOVA on mean RTs in the N-Back task revealed significant main effects of Time [$F(1, 35) = 58.100$, $p < .001$, $\eta^2_p = .620$] and Workload [$F(2, 70) = 26.340$, $p < .001$, $\eta^2_p =$

565 .430]. Lower RTs were observed during-tDCS (1456.930 ± 17.430 ms) than at pre-tDCS
566 (1474.120 ± 17.360 ms), reflecting a decrease in RTs over time. Additionally, RTs differed
567 across workload conditions, with lower RTs in the 0-back condition (1399.800 ± 15.600 ms)
568 than in the 1-back (1477.310 ± 21.310 ms) and 2-back conditions (1519.470 ± 22.050 ms). No
569 other main effects or interactions reached significance (all Fs < 2.400, all *ps* > .130).

570

571 **Table 3. Means (± SEM) for reaction times (ms) by Time, Stimulation, and Workload.**

Time	Stimulation	0-back (ms)	1-back (ms)	2-back (ms)
Pre	Sham	1398.250 ± 22.320	1463.720 ± 27.370	1503.830 ± 26.980
Pre	Anodal	1415.110 ± 17.350	1511.250 ± 26.970	1552.560 ± 26.910
During	Sham	1379.560 ± 22.830	1444.310 ± 27.560	1483.920 ± 26.770
During	Anodal	1406.280 ± 16.910	1489.940 ± 28.000	1537.560 ± 28.190

572 **Note.** Values represent mean reaction times (± SEM) in milliseconds for the N-Back task. Pre-
573 tDCS = baseline (N-Back task performed without HD-tDCS stimulation); during-tDCS = during
574 stimulation (tasks performed under sham or anodal HD-tDCS). Workload levels correspond to
575 the three N-Back conditions: 0-back, 1-back, and 2-back. SEM = standard error of the mean.

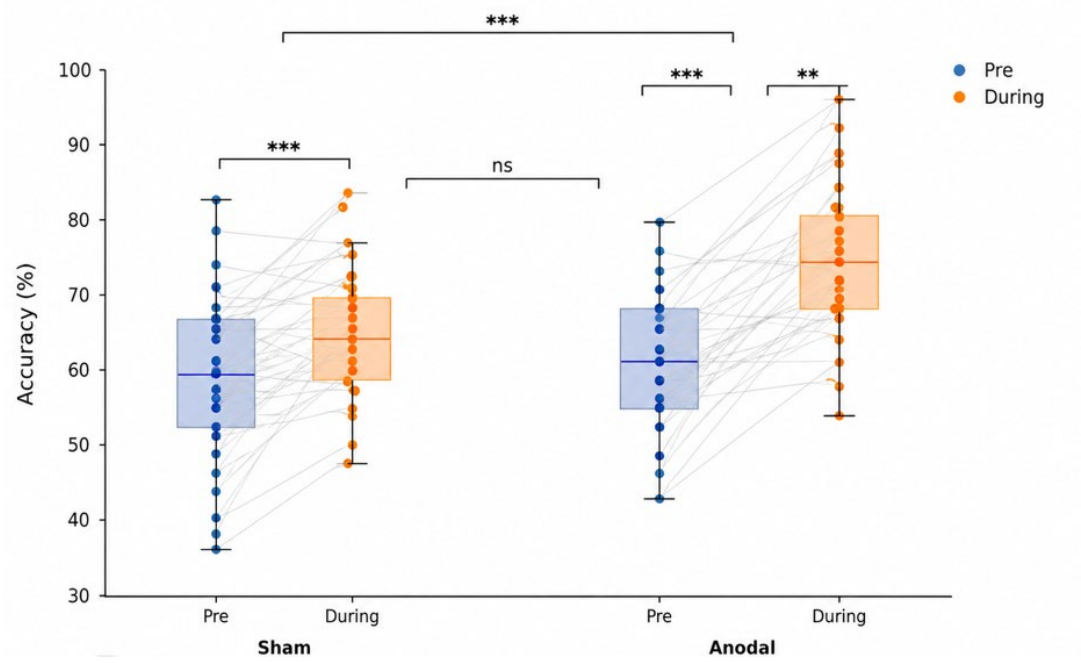
576 Taken together, RTs differed across time points and task workload levels, whereas no
577 significant main effects or interactions involving Stimulation were observed.

578 ## Accuracy

579 The 3-way ANOVA on mean accuracy in the N-Back task revealed a significant Time ×
580 Stimulation interaction [$F(1, 35) = 17.265$, $p < .001$, $\eta^2_p = .330$; see Fig. 5]. Post hoc
581 comparisons showed that accuracy improved significantly from pre-tDCS to during-tDCS under
582 both sham stimulation (58.981% ± 1.525 vs. 64.722% ± 1.545; $p < .001$) and anodal stimulation

(61.204% ± 1.521 vs. 73.889% ± 1.749; $p < .001$). Accuracy did not differ significantly between sham and anodal stimulation at pre-tDCS (58.981% ± 1.525 vs. 61.204% ± 1.521; $p = .260$), whereas during-tDCS it was significantly higher under anodal than sham stimulation (73.889% ± 1.749 vs. 64.722% ± 1.545; $p < .001$). Thus, although accuracy increased over time in both conditions, the observed increase was greater under anodal than sham stimulation. For completeness, significant main effects of Time [$F(1, 35) = 115.628$, $p < .001$, $\eta^2_p = .768$], Workload [$F(2, 70) = 165.561$, $p < .001$, $\eta^2_p = .825$], and Stimulation [$F(1, 35) = 25.327$, $p < .001$, $\eta^2_p = .420$] were also observed. Overall, accuracy was higher during-tDCS than at pre-tDCS (69.306% ± 1.460 vs. 60.093% ± 1.390), decreased with increasing workload (0-back: 78.264% ± 1.390; 1-back: 67.361% ± 1.530; 2-back: 48.472% ± 1.930; all ps < .001), and was higher during anodal than sham stimulation (67.546% ± 1.520 vs. 61.852% ± 1.420; $p < .001$). No other main effects or interactions reached significance (all Fs < 1.225, all ps > .300).

Figure 5. N-Back accuracy during sham and anodal HD-tDCS over the left dorsolateral prefrontal cortex.



Note. Individual participant accuracy values are shown, with paired observations connected

across time points. Boxplots show the median (horizontal line), interquartile range (box), and whiskers extending to values within 1.5 × the interquartile range. Each dot represents an individual participant, and grey lines connect paired observations across time points within each stimulation condition. Accuracy values are averaged across the three N-Back workload conditions (0-, 1-, and 2-back). Pre-tDCS = baseline; during-tDCS = task performance during sham or anodal HD-tDCS. **p < .01; ***p < .001; ns = non-significant.

Taken together, the N-Back findings indicate that anodal HD-tDCS was associated with a greater within-session improvement in working-memory accuracy than sham stimulation.

Correlation analyses: HF-HRV and cognitive outcomes

Pearson correlation analyses revealed that during anodal stimulation of the left DLPFC, significant associations emerged between HF-HRV and changes in cognitive performance (Δ scores: during-tDCS – pre-tDCS) on both the Stroop and N-Back tasks. Specifically, in the anodal condition, HF-HRV was negatively correlated with Stroop task reaction times ($r = –.461$, $p = .020$), indicating that higher parasympathetic activity was associated with faster responses, consistent with a relationship between autonomic regulation and inhibitory control. This pattern is consistent with an association between parasympathetic activity and inhibitory-control performance. In contrast, no significant associations were observed between HF-HRV and Stroop performance changes in the sham condition (all $ps > .200$).

For the N-Back task, under anodal stimulation, HF-HRV was positively correlated with accuracy ($r = .490$, $p = .006$) and negatively correlated with RTs ($r = –.452$, $p = .023$), indicating that greater HF-HRV was associated with enhanced working memory performance, reflected in both higher accuracy and faster responses. This pattern is consistent with an association between parasympathetic activity and working-memory performance. In contrast, no significant associations were found between HF-HRV and N-Back performance changes in the sham

condition (all *ps* > .05).

Overall, these findings indicate that under anodal HD-tDCS, HF-HRV was significantly associated with changes in executive-task performance, whereas no significant associations were observed under sham stimulation.

Discussion

To the best of our knowledge, this is the first study to investigate, within the Neurovisceral Integration framework (NIM; Thayer & Lane, 2000; Thayer et al., 2009), the concurrent associations between anodal HD-tDCS over the left DLPFC, executive performance, affective responses, and autonomic regulation in healthy adults. Our findings indicated that anodal HD-tDCS over the left DLPFC was associated with higher working-memory accuracy and with subtle differences in the pattern of Stroop reaction times over time. Moreover, anodal stimulation was accompanied by a greater increase in HF-HRV than sham stimulation, consistent with higher parasympathetic activity during task performance. However, no significant stimulation-related differences were observed in self-reported affective states as measured by the PANAS.

Overall, the Stroop results indicated changes in performance over time across stimulation conditions. Reaction times decreased from pre- to during-tDCS in both sessions, with a significantly greater reduction observed in the anodal than in the sham condition, as reflected by the significant Time × Stimulation interaction. However, reaction times did not differ between stimulation conditions during the tDCS phase. Supplementary analyses further indicated that the magnitude of the pre-to-during RT change was greater under anodal than sham stimulation, whereas baseline RT was not significantly associated with the magnitude of RT change in either condition, providing no evidence that the differential change was simply attributable to baseline-dependent improvement.

With respect to accuracy, participants remained less accurate on incongruent than

congruent trials across both phases, consistent with a robust interference effect. Accuracy increased from pre- to during-tDCS for both congruent and incongruent trials, a pattern that may partly reflect practice-related facilitation. Although numerically higher accuracy was observed during the anodal condition, the differential stimulation effect on accuracy was modest and should be interpreted cautiously. Accordingly, these findings should not be taken as evidence of improved inhibitory control. Rather, the overall pattern may be consistent with subtle stimulation-related differences in cognitive-control processes during conflict processing, although the underlying mechanisms were not directly assessed in the present study (e.g., Abedanzadeh et al., 2021).

The pattern observed for accuracy is broadly consistent with Perrotta et al. (2021), who reported reduced error rates in incongruent Stroop trials following anodal stimulation over the left DLPFC. In contrast, Frings et al. (2018) reported that cathodal stimulation over the left DLPFC was associated with increased error rates in incongruent trials relative to anodal stimulation. However, their study lacked a sham control condition and employed a modified Stroop paradigm with a larger number of trials, which may limit direct comparison with the present findings. In addition, compared with the focal 4 × 1 HD montage used in the present study, Frings et al. (2018) employed a conventional electrode configuration (9 cm^2 anode over the left DLPFC and a 35 cm^2 reference electrode over the parieto-occipital cortex), which may have resulted in broader current spread and lower focality at the target site (Datta et al., 2008; Saturnino et al., 2015).

Regarding the N-Back task, anodal HD-tDCS was associated with higher accuracy during the stimulation phase compared with sham, consistent with previous evidence linking DLPFC modulation to working-memory performance (Fregni et al., 2005; Hill et al., 2016). The significant Time × Stimulation interaction indicated that the increase in accuracy from pre- to during-tDCS differed between stimulation conditions, with a greater increase observed under anodal than sham stimulation. This pattern may be consistent with differential engagement of

cognitive-control processes involved in the updating and maintenance of information, although the underlying mechanisms were not directly assessed in the present study. Performance nevertheless declined progressively from the 0-back to the 2-back condition, reflecting the increasing cognitive demands imposed by higher levels of working-memory load. Importantly, the absence of a Workload × Stimulation interaction suggests that the association between stimulation condition and performance was not selectively stronger at a specific level of working-memory demand.

The greater increase in accuracy observed in the present study contrasts with the pattern reported by Brunoni and Vanderhasselt (2014), whose meta-analysis suggested that anodal tDCS over the DLPFC may be more consistently associated with changes in reaction time than with accuracy in working-memory tasks. This discrepancy may reflect differences in stimulation protocols, electrode configurations, task parameters, or sample characteristics across studies, highlighting the complexity of tDCS-related outcomes and the importance of contextual factors in shaping cognitive performance. Although stimulation was delivered in a single session concurrently with task performance, the observed pattern is consistent with a possible association between brief HD-tDCS exposure and short-term changes in working-memory performance (Fregni et al., 2005; Mulquiney et al., 2011). One possible interpretation is that the observed pattern may be compatible with changes in DLPFC excitability and corresponding differences in the recruitment of frontal control networks during task execution (Nitsche & Paulus, 2000; Nitsche et al., 2008; Chan et al., 2021); however, these neurophysiological mechanisms were not directly assessed in the present study.

In addition to the observed cognitive changes, differences in autonomic activity were also observed. Specifically, the increase in HF-HRV during anodal stimulation is consistent with greater parasympathetic activity, which has previously been associated with executive functioning (Makovac et al., 2017; Schmaußer et al., 2022). Taken together, these concurrent cognitive and autonomic patterns are compatible with the Neurovisceral Integration Model

(NIM; Thayer & Lane, 2000; Thayer et al., 2009; Smith et al., 2017), which proposes that prefrontal–subcortical circuits contribute jointly to cognitive and autonomic regulation

The concurrent increase in HF-HRV and higher working-memory accuracy under anodal HD-tDCS may reflect a relationship between executive functioning and parasympathetic regulation. Although no significant improvement was observed in inhibitory control accuracy, this pattern is broadly consistent with previous evidence linking higher vagally mediated HRV to superior executive functioning (Forte et al., 2021; Luque-Casado et al., 2016). Accordingly, these findings should not be interpreted as evidence of improved inhibitory control, but rather as indicating an association between autonomic regulation and selected aspects of executive functioning. This interpretation is consistent with previous reports showing concurrent changes in vagally mediated autonomic regulation and cognitive performance during prefrontal stimulation (Nikolin et al., 2017; Schmaußer et al., 2022). The increase in HF-HRV observed during task performance under anodal HD-tDCS over the DLPFC is also consistent with previous findings (Nikolin et al., 2017) and suggests that autonomic and cognitive changes may occur concurrently during left DLPFC stimulation. More broadly, this pattern is compatible with emerging evidence that HRV may change following a single session of anodal tDCS (Schmaußer et al., 2022).

Additional findings broadly consistent with the NIM emerged from the significant correlations observed in the anodal condition, whereby higher HF-HRV was associated with faster RTs on the Stroop task and with both higher accuracy and faster RTs on the N-Back task. These associations suggest a possible relationship between autonomic regulation and performance across selected executive tasks, particularly working memory and conflict-processing performance. Comparable associations were not observed in the sham condition. However, because the strength of the correlations was not formally compared across stimulation conditions, this pattern should be interpreted cautiously and cannot be considered evidence of a condition-specific association.

It should be noted that previous research examining the relationship between tDCS and HRV has yielded mixed results. For example, Gu et al. (2022) reported that anodal HD-tDCS over the left DLPFC applied at rest (i.e., without a concurrent task) was associated with higher indices of sympathetic activity (higher LF power and LF/HF ratio), rather than parasympathetic activation. In contrast, Vandermeeren et al. (2010) found no significant changes in HRV when stimulation was applied over the midline frontal cortex with an extracephalic reference electrode. Consistent with this variability, a recent systematic review by Schmaußer et al. (2022) reported small-to-medium effect sizes for associations between brain stimulation and HRV outcomes. Notably, the studies included in that review largely differed in stimulation montages, task contexts (rest vs. active engagement), and HRV indices, which may contribute to variability in the reported findings. Taken together, the available evidence suggests that heterogeneity across studies may be related to a combination of contextual and methodological factors, including stimulation parameters, cognitive demands, and physiological measurement approaches (Shaffer & Ginsberg, 2017; Vergallito et al., 2022). Future research should continue to systematically examine these variables when investigating associations between tDCS and autonomic regulation.

Lastly, the absence of significant differences in self-reported affect across stimulation conditions, as measured by the PANAS, aligns with previous findings reporting limited mood effects following a single session of prefrontal stimulation in healthy individuals (Nitsche et al., 2012; Remue et al., 2016). Thus, the cognitive and autonomic effects observed in the present study were not accompanied by significant changes in subjective affect.

Limitations

One limitation of the present study is that autonomic regulation was assessed exclusively using HF-HRV. HF-HRV was selected a priori as the primary autonomic outcome because it is a well-established index of cardiac vagal activity and the HRV measure most closely aligned with the NIM framework (e.g., Pawling et al., 2024; Thayer et al., 2009) and previous HD-tDCS

research (Nikolin et al., 2017). Accordingly, the study was designed to test hypotheses specifically related to HF-HRV rather than to provide a comprehensive characterisation of autonomic function. Although additional HRV metrics (e.g., RMSSD or SDNN) may provide complementary information, they were not prespecified outcomes. Furthermore, the raw inter-beat interval files and complete Kubios outputs required to derive these indices retrospectively were no longer available, precluding their reliable calculation using the same analytical pipeline adopted for the primary outcome. Future studies incorporating a broader autonomic assessment will be important to determine whether similar patterns are observed across HRV indices.

Despite counterbalancing procedures, the repeated administration of cognitive tasks within a single session may have introduced practice effects, potentially contributing to changes in performance independently of stimulation condition. Although differential changes were reflected in significant interaction effects, some aspects of the observed patterns may also have been influenced by baseline variability across sessions. Future studies employing alternate task versions or longer washout intervals may help to more clearly distinguish practice-related changes from differences associated with stimulation condition.

Another limitation of the present study is the relatively wide age range of the sample (19–75 years). Age-related differences in cortical structure, neuroplasticity, and baseline cognitive functioning may be associated with variability in responses to HD-tDCS (Fujiyama et al., 2014; Ghasemian-Shirvan et al., 2022; Indahlastari et al., 2021). To explore this issue, additional linear regression analyses were conducted using age as a continuous predictor of the differential stimulation effect (ΔΔ) for HF-HRV, Stroop reaction time, Stroop accuracy, N-Back reaction time, and N-Back accuracy. None of these associations reached statistical significance (all $p \geq .417$; Supplementary Table S4). However, the present study was designed and powered to investigate within-subject stimulation effects rather than age-related moderation effects, and these exploratory null findings should therefore be interpreted cautiously. Future studies with larger and more age-balanced samples should specifically examine age as a potential moderator

of cognitive and autonomic responses observed under HD-tDCS.

Finally, stimulation targeted exclusively the left DLPFC using a 4 × 1 HD montage. While this configuration is designed to provide greater focality (Datta et al., 2008), it limits generalisability to right hemispheric or bilateral stimulation protocols. Future research should examine potential lateralisation effects and compare focal with conventional montages to clarify whether stimulation-related patterns vary according to hemispheric target and electrode configuration.

Conclusion

In summary, the present findings indicate that anodal HD-tDCS targeting the left DLPFC was associated with higher working-memory accuracy, differences in executive-task performance, and increased HF-HRV during task engagement. The association observed between HF-HRV and cognitive outcomes under anodal stimulation may reflect a relationship between autonomic regulation and executive performance. Overall, the findings suggest that cognitive-control performance and neurocardiac regulation may vary concurrently during anodal HD-tDCS over the left DLPFC.

These preliminary findings warrant further investigation of HD-tDCS in healthy individuals. Future studies should examine the reproducibility and persistence of these patterns, clarify the mechanisms that may underlie them, and determine whether similar patterns are observed in clinical populations characterised by executive and autonomic dysregulation, such as Parkinson's disease and Tourette syndrome.

Author Contributions

Conceptualisation, Methodology, Software, Assessment, Data collection, Data Curation, Visualisation: [name]. Writing—Original Draft, Formal Analysis, Validation: [name], [name]. Writing—Review & Editing: [name]. Supervision: [name].

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Conflict of Interest Statement

The authors declare no conflicts of interest.

Funding information

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Data Accessibility

The dataset supporting the findings of this study is publicly available on the Open Science Framework (OSF) at [link]. The repository contains the final dataset corresponding to the analyses reported in the present manuscript. Earlier exploratory variables not included in the reported analyses have been removed to ensure consistency with the submitted version.

Declaration of AI-assisted technologies in the manuscript preparation process

During the preparation of this manuscript, the authors used an AI-based language model to improve language clarity and readability. After using this tool/service, the authors reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

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Supplemental material

Blinding assessment

At the end of each session, participants were asked to indicate whether they believed they had received anodal or sham stimulation to evaluate the effectiveness of sham blinding (Stanković et al., 2021). In the first visit, 33% of participants correctly identified the stimulation condition, whereas 41% did so in the second visit. Binomial tests indicated that the proportion of correct guesses did not differ significantly from chance level (50%) at either visit (all $p > .05$), suggesting that blinding was preserved.

To assess whether perceived stimulation condition influenced behavioural outcomes, we computed a speed–accuracy trade-off index for both the Stroop and N-Back tasks. Independent-samples comparisons between participants who correctly guessed the stimulation condition and those who did revealed no evidence of performance differences (two-sided *p*-values ranging from .28 to .95). These findings indicate that task performance was not associated with participants' beliefs about stimulation type.

Table S1. Pre-tDCS Baseline Comparisons Between Sham and Anodal Sessions

Baseline outcome	Sham Mean ± SD	Anodal Mean ± SD	*p*	Bonferroni-adjusted *p*	Cohen's *d*
HF-HRV					

Baseline outcome	Sham Mean ± SD	Anodal Mean ± SD	*p*	Bonferroni-adjusted *p*	Cohen's *d*
Stroop	50.830 ± 9.475	51.478 ± 9.156	.102	.612	0.280
N-Back	51.137 ± 9.169	52.815 ± 8.987	.019	.114	0.411
Stroop task					
RT (ms)	1076.708 ± 166.652	1084.181 ± 152.354	.726	1.000	0.059
Accuracy (%)	80.778 ± 5.962	81.306 ± 6.610	.649	1.000	0.077
N-Back task					
RT (ms)	1492.972 ± 122.324	1455.269 ± 135.319	.146	.876	0.248
Accuracy (%)	58.982 ± 9.148	61.204 ± 9.125	.081	.486	0.299

Note. Values are presented as mean ± SD. HF-HRV = high-frequency heart rate variability; baseline HF-HRV represents values obtained during the pre-tDCS phase and is reported separately for the Stroop and N-Back task periods. Stroop reaction time represents the mean across congruent and incongruent trials, and accuracy represents the percentage of correct responses across Stroop trials. N-Back reaction time represents the mean across the 0-, 1-, and 2-back conditions, and accuracy represents the percentage of correct responses averaged across the three workload conditions. Sham and anodal pre-tDCS values were compared using two-tailed paired-samples t-tests. Bonferroni-adjusted p values were calculated across the six physiological and cognitive baseline comparisons. Cohen's d was calculated using the standard deviation of the paired differences. SD = standard deviation; RT = reaction time.

Summary: Supplementary baseline comparisons indicated that none of the physiological or cognitive measures differed significantly between the sham and anodal sessions after Bonferroni correction (all adjusted $p \geq .114$). Although the unadjusted comparison for HF-HRV

during the N-Back task reached significance (p = .019), this difference did not remain significant after correction ($p$adjusted = .114).

Table S2. Comparison of Stroop reaction time-change (ΔRT) scores between sham and anodal

Condition	ΔRT Mean ± SD (ms)	Mean difference	95% CI	t(35)	p	Cohen's d
Sham	−9.750 ± 17.067					
Anodal	−18.903 ± 11.502	**9.153**	**[3.077, 15.229]**	**3.058**	**.004**	**0.510**

Note. ΔRT was calculated as the difference between during-tDCS RT and pre-tDCS RT; therefore, negative values indicate faster reaction times during stimulation relative to baseline. Stroop RT represents the mean reaction time across congruent and incongruent trials. The mean difference represents ΔRT Sham − ΔRT Anodal. The comparison was performed using a two-tailed paired-samples t-test. CI = confidence interval; RT = reaction time; SD = standard deviation.

Summary: Stroop reaction times decreased from pre- to during-tDCS in both stimulation conditions, but the reduction was significantly greater in the anodal condition than in the sham condition, t(35) = 3.058, unadjusted p = .004, Bonferroni-adjusted p = .012, Cohen's d = 0.510. This indicates a larger pre-to-during reduction in RT under anodal stimulation.

Table S3. Correlation between baseline Stroop reaction time and change (ΔRT) in reaction time

Stimulation condition	Pearson's r	p
Sham	**−.072**	**.677**
Anodal	**.127**	**.462**

Note. Pearson correlations examined the association between baseline Stroop RT and ΔRT separately within each stimulation condition. ΔRT was calculated as during-tDCS RT minus pre-tDCS RT. RT = reaction time

Summary: Baseline Stroop reaction time was not significantly associated with ΔRT in either the sham condition (r = −.072, p = .677) or the anodal condition (r = .127, p = .462).

Thus, there was no evidence that participants with slower baseline responses systematically showed larger subsequent reductions in RT.

Table S4. Linear Regression Analyses Examining Age as a Predictor of Differential Stimulation Effects

Outcome	B (Age)	SE	β	R^2	F(1, 34)	p
HF-HRV	0.020	0.027	0.127	0.016	0.560	.460
Stroop RT	0.115	0.140	0.140	0.019	0.675	.417
Stroop Accuracy	−0.002	0.020	−0.018	< .001	0.011	.918
N-Back RT	−0.079	0.206	−0.066	0.004	0.148	.703
N-Back Accuracy	0.034	0.079	0.074	0.005	0.188	.667

Note. For each outcome, change scores (Δ) were calculated separately for the anodal and sham conditions as during-tDCS minus pre-tDCS. Differential change scores (ΔΔ) were then calculated as ΔAnodal minus ΔSham. Separate linear regression analyses were performed for each outcome, with ΔΔ as the dependent variable and age (years) as the continuous predictor. HF-HRV represents high-frequency heart rate variability averaged across the Stroop and N-Back task periods. Stroop outcomes were averaged across congruent and incongruent trials, whereas N-Back outcomes were averaged across the 0-, 1-, and 2-back conditions. B = unstandardized regression coefficient; SE = standard error; β = standardised regression coefficient; R^2 = proportion of variance in the differential stimulation effect explained by age; RT = reaction time.

Summary**:** Age was not significantly associated with the differential stimulation effect for HF-HRV (B = 0.020, p = .460), Stroop RT (B = 0.115, p = .417), Stroop accuracy (B = −0.002, p = .918), N-Back RT (B = −0.079, p = .703), or N-Back accuracy (B = 0.034, p = .667). Across outcomes, age explained less than 2% of the variance in the differential stimulation effect ($R^2 \leq .019$). These exploratory analyses therefore did not provide evidence that the magnitude of the differential pre-to-during stimulation effect systematically varied as a function of age within the present sample. Given that the study was not designed or powered to examine age-related moderation, these null findings should be interpreted cautiously.