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RESEARCH REPORT

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Responsiveness to left-prefrontal tDCS varies according to arousal levels

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Abstract

Over the past two decades, the postulated modulatory effects of transcranial direct current stimulation (tDCS) on the human brain have been extensively investigated. However, recent concerns on reliability of tDCS effects have been raised, principally due to reduced replicability and to interindividual variability in response to tDCS. These inconsistencies are likely due to the interplay between the level of induced cortical excitability and unaccounted structural and state-dependent functional factors. On these grounds, we aimed at verifying whether the behavioural effects induced by a common tDCS montage (F3-rSOA) were influenced by the participants' arousal levels, as part of a broader mechanism of state-dependency. Pupillary dynamics were recorded during an auditory oddball task while applying either a sham or real tDCS. The tDCS effects were evaluated as a function of subjective and physiological arousal predictors (STAI-Y State scores and pre-stimulus pupil size, respectively). We showed that prefrontal tDCS hindered task learning effects on response speed such that performance improvement occurred during *sham*, but not *real* stimulation. Moreover, both subjective and physiological arousal predictors significantly explained performance during real tDCS, with interaction effects showing performance improvement only with moderate arousal levels; likewise, pupil response was affected by real tDCS according to the ongoing levels of arousal, with reduced dilation during higher arousal trials. These findings highlight the potential role of arousal in shaping the neuromodulatory outcome, thus emphasizing a more careful interpretation of null or negative results while also encouraging more individually tailored tDCS applications based on arousal levels, especially in clinical populations.

KEYWORDS
pupil, response variability, state dependency, tDCS

Abbreviations: LC, locus coeruleus; LMMs, linear mixed models; PD, pupil dilation; PrePD, pre-stimulus pupil diameter; RT, reaction times; STAI-Y, State–Trait Anxiety Inventory; tDCS, transcranial direct current stimulation.

1 | INTRODUCTION

Founded on decades of experimentation, transcranial direct current stimulation (tDCS) is a research tool capable of interacting with the central nervous system that has been rediscovered at the beginning of this century (Priori, 2003). Beside its value for basic research (Antal et al., 2017), tDCS has raised great interest for real-world applications, like rehabilitative interventions for neurological and psychiatric diseases (Lefaucheur et al., 2017) and cognitive enhancement (or detraction) in both young and older adults (Ke et al., 2019; Nelson et al., 2016; Santarnecchi et al., 2015; Summers et al., 2016). However, our incomplete understanding on how the physiological effects link to a behavioural outcome has hindered the development of more effective and generalizable stimulation protocols (Bestmann et al., 2015). What raises most concern is the lack of replicability among studies and the interindividual variability in response to tDCS (Horvath et al., 2015a,b; Medina & Cason, 2017; Wiethoff et al., 2014). In addition to non-optimal methodological practices, a complex interplay among brain anatomical differences (i.e., cortical surface topography, skull thickness, subcutaneous fat levels and cerebrospinal fluid density) and the level of neuromodulatory effects might be crucial in explaining the inconsistencies across studies. Furthermore, state-based factors, including the levels of activation prior and during stimulation, the performance rank, wakefulness, task priming or novelty, might all play a decisive role (Horvath et al., 2014; Krause & Kadosh, 2014; Li et al., 2015); likewise, trait-based differences that strictly depend on neurotransmitter systems, such as different degrees of impulsive and risky behaviour, are also key to understanding the potential negative effects of tDCS (Bell et al., 2020). As a result, it appears conceivable to interpret the final effects of tDCS as contingent on the level of network engagement (Fertonani & Miniussi, 2017; Miniussi et al., 2013). In line with this prediction, many experiments have demonstrated a clear effect of baseline levels of different mental capabilities on tDCS-induced response (Benwell et al., 2015; Berryhill & Jones, 2012; Hsu et al., 2016; Mauri et al., 2015; Sarkar et al., 2014). Most recently, variability in the context of prefrontal tDCS has been associated with GABA- and glutamate-driven levels of excitability of the targeted cortex (Filmer, Ehrhardt, Bollmann, et al., 2019).

Further concerns have been raised in the field of transcranial electric stimulation regarding the subcutaneous nerves stimulation that may contribute even unconsciously to the final behavioural outcome (Liu et al., 2018). The possibility of indirectly affecting brain circuits likely adds on the participant's initial state- or trait-based levels that make these measurements even more important for a better interpretation of the end results.Importantly, the use of tDCS is known to have an impact on large-scale brain systems extending well beyond the area under the stimulating electrode (Antal et al., 2011; Sandrini et al., 2020; Wagner et al., 2014; Weber et al., 2014). This approach translates into a lack of focality that resembles the spread of the noradrenergic modulatory action exerted by the locus coeruleus (LC), which arguably subtends arousal functions. This midbrain system is widely recognized for its role in shaping behavioural performance of primates (Aston-Jones et al., 1999; Berridge & Waterhouse, 2003; Sara & Bouret, 2012). A large body of evidence also suggests that the exogenous direct currents and the endogenous modulatory action on target cells share the same central mechanism of neuronal gain control (Aston-Jones & Cohen, 2005; Lafon et al., 2017; Moxon et al., 2007; Servan-Schreiber et al., 1990). Therefore, an interrelation between the two stimulating activities seems reasonable to the extent that whenever the contrast between activated and inhibited units becomes sufficiently increased or decreased any further added neuromodulation can likely spoil the expected results. In this respect, the human LC endogenous action measured by pupil dilation during response inhibition processes was shown to depend on whether basal neural excitability was modulated or not prior to task completion (Adelhöfer et al., 2019). Furthermore, a widespread release of noradrenaline could selectively interact with the local glutamate-driven levels of excitability, which have been described as generating feedback loops in tasks with different degrees of stimulus priority (see the GANE model, Mather et al., 2016). To this regard, we have recently showed that existing information-priority advantage of high saliency stimuli was differently modulated when applying transcranial electric stimulation in young and elderly individuals, according to their arousal levels at baseline (Esposito et al., 2021).

Given the above considerations, it appears evident that great part of the tDCS behavioural variability may stem from the interdependency between the induced cortical excitability and the varying levels of arousal experienced by participants. The aim of this study was to verify whether the behavioural and physiological responses induced by tDCS were somehow dependent on the participants' arousal levels. We chose a broadly used tDCS montage in the neuromodulation literature (e.g., Dedoncker et al., 2016; Lefaucheur et al., 2017) that supposedly target the prefrontal cortex in basic cognitive protocols (e.g., Mulquiney et al., 2011; Penolazzi et al., 2013; Plewnia et al., 2013; Sandrini et al., 2014; Savic et al., 2017), as well as in clinical trials

(e.g., Palm et al., 2012; Smith et al., 2015; Suemoto et al., 2014).

The tDCS was applied during an auditory oddball task aimed to probe cognitive performance as a function of arousal levels (Beatty, 1982) and purposefully designed to keep participants' attention on task over uncertain periods (i.e., variable instead of fixed inter stimulus interval) in a way that online tDCS effects would be more subjected to a higher volatility of sustained attention (Langner & Eickhoff, 2013; Unsworth et al., 2018).

We tracked pupillary changes as a proxy for the LC modulatory action (Costa & Rudebeck, 2016; Murphy et al., 2011). We used reaction times (RT) and pupil dilation (PD) as a measure of LC phasic response to the relevant stimuli (target) and pre-stimulus pupil diameter (PrePD) as a physiological marker of the LC tonic discharge activity. Subjective arousal levels were also evaluated by means of State–Trait Anxiety Inventory (STAI-Y), an assessment tool for both trait and state factors relating to anxiety, from which we used state-related scores (Spielberger, 2010).

2 | MATERIALS AND METHODS

2.1 | Participants

Eighteen right-handed healthy participants (mean [SD] age = 23.7 [3.8]; 10 females; mean [SD] STAI-Y trait score = 44.9 [3.9]) took part in the experiment. Participants had no history of neurological or psychiatric illness and had normal or corrected-to-normal visual acuity. Ethical approval was obtained by the Ethics Committee of the IRCCS Centro San Giovanni di Dio Fatebenefratelli, Brescia, Italy. All participants were given written informed consent.

2.2 | Experimental design and procedure

A single-blind within-subject design was implemented. The testing sessions were organized in two days separated by at least 48 h in order to exclude any tDCS carry-over effects. In each session, participants completed the task twice: at *baseline* (T1) without any electrodes mounted on their scalp and subsequently either during *sham* or *real* stimulation (T2) (Figure 1b). We collected behavioural and pupil data for the whole task duration (∼18 min).

Participants were randomly assigned and counterbalanced across two session-orders of tDCS protocol, and they were kept blind to the ongoing experimental condition (i.e., sham or real). The same participant was tested at around the same hour to control for any arousal variation due to the daily metabolic cycle and circadian rhythms (Oken et al., 2006). Moreover, we controlled that all participants were balanced in terms of amount of sleep, caffeine, alcohol and nicotine consumption. Participants seated in a soundproof dark room at the distance of about 55 cm from a 17-in LCD monitor and with the only source of light provided by a grey fixation cross. The auditory oddball task was presented using E-Prime presentation software (Schneider et al., 2001) by means of two constant-loudness speakers (Figure 1a).

In every task condition, there was a fixed total number of trials (420) of which 20% included targets (84) and 80% standards stimuli (336). The interstimulus interval was set to a range of 2.1–2.9 s and both stimuli lasted for 70 ms. In so doing, we ensured enough time (∼8 s) for the phasic pupil dilation to return to baseline before overlapping to the next target trial, that is, by playing at least three non-target tones between a target and the next one (Gilzenrat et al., 2010; Murphy et al., 2011). Along with a short training session, participants were instructed to readily press a button with their right index finger whenever detecting a target tone and to keep their gaze on the fixation cross throughout the task. Speed of response and gaze fixation were emphasized before each task execution. At the end of each experimental session, participants were given a questionnaire to rate the perceived sensations or discomforts that influenced their performance (Fertonani et al., 2015).

2.3 | tDCS protocol

A battery-driven current stimulator (Brain-STIM, EMS, Bologna, Italy) was used to deliver 1-mA (0.028 mA/cm^2) direct current stimulation via two rubber electrodes (35 cm^2) which were inserted inside two saline-soaked sponges. In order to ensure a stable impedance level as well as keeping skin sensations at the minimum, conductive electro-gel was also applied.The electrodes montage consisted in placing the anode over the area F3 of the EEG 10–20 system and the return (cathode) electrode over the right supra-orbital area (Figure 1c). The duration of the stimulation consisted of about 17 min (1040 s) with 15 s of currents fade-in and fade-out. Configuration of the sham condition included 15 s of fade-in, 10 s of actual current delivery and 15 s of fade-out given at the beginning of the experiment only (Figure 1d).

Mindful that the mere sensory stimulation could confound the expected arousal effects, we run a pilot

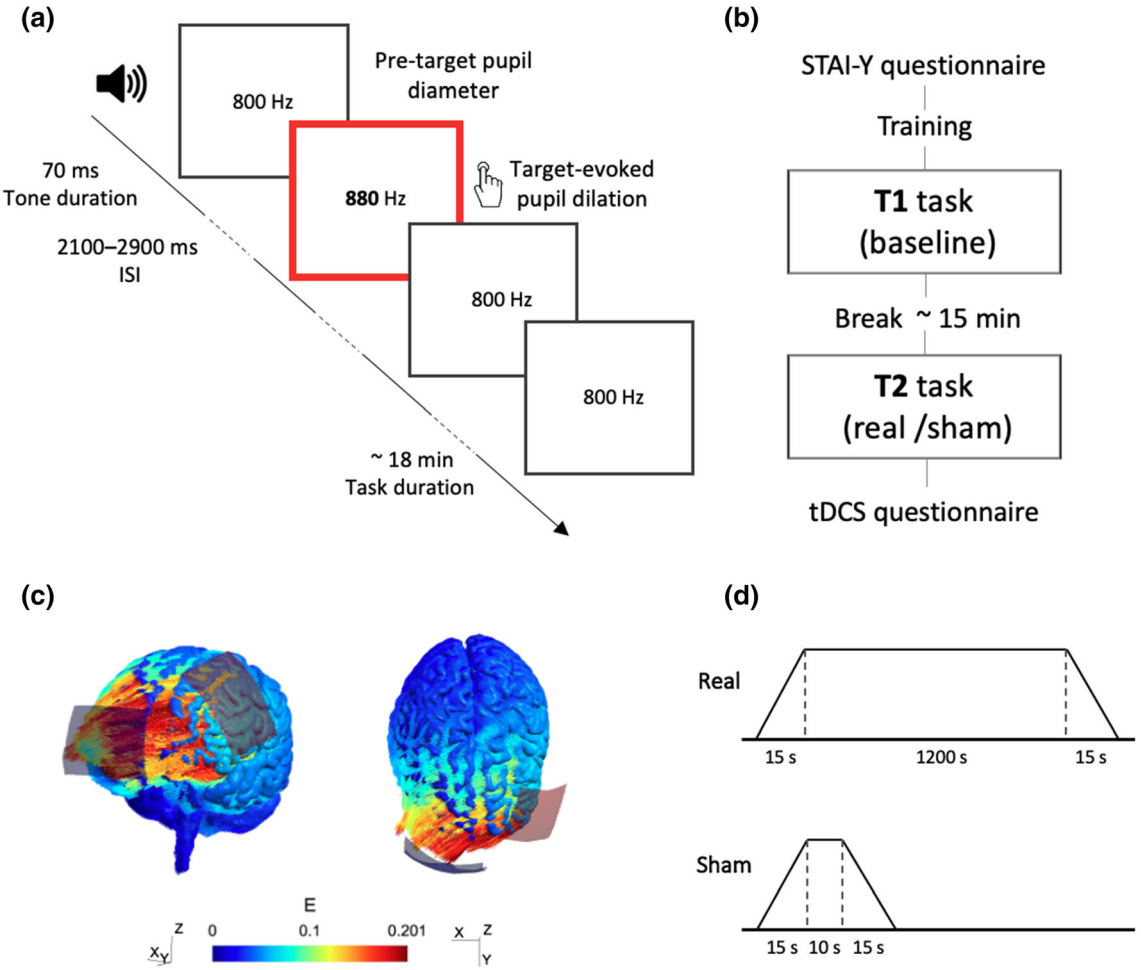

FIGURE 1 Study design and task paradigm. (a) Example of a trial sequence. (b) Overview of the experimental timeline, showing two testing sessions each one with two task conditions: baseline and stimulation. (c) Simulation results for the applied tDCS montage and parameters using SimNIBS toolbox (Saturnino et al., 2019). The colours denote the strength of the electric fields (independently of current flow directionality) simulated in a default head model. (d) Schematic representation of the stimulation protocol

experiment to validate our blind-controlled tDCS protocol and to exclude unexpected technical concerns due to the use of eye-tracker and the simultaneous current delivery.

Ten right-handed healthy participants were recruited prior to the main study (mean age [SD] = 26.4 [1.7]; 6 females; mean [SD] STAI-Y trait score = 44.7 [2.3]). All participants underwent, in a counterbalanced order, real and sham tDCS at rest in the same day, interposed by a pause of 15 min. Each tDCS protocol lasted about 5 min, a duration whose excitability modulations have been proved to return to baseline within the following 5 min (Nitsche & Paulus, 2000, 2001). We showed that neither participants were subjectively aware of the type of stimulation (i.e., sensation questionnaire and oral report) nor did their rate of full or half-eye blinks, known to tap into the dopaminergic and fatigue-related neural pathways (Stern et al., 1994), differ between sham (8.07% ± 5.8) and real stimulation (7.76% ± 5.9) ($t_{(9)} = 0.47$; $p = 0.64$). Despite the limited sample size, we deemed this evidence supportive for our within-subject study design.

2.4 | Pupil signal recording and pre-processing

Pupil diameter was recorded with an EyeLink 1000 Plus system (SR Research, Osgood, ON, Canada) and at 500-Hz sampling rate with left-monocular and pupil-CR tracking mode. Pupil signal was processed offline. A shape-preserving piecewise cubic interpolation method was chosen to interpolate values ranging from 70 ms before blink onset to 300 after blink offset. Epoch

segmentation and baseline correction were then carried out (respectively for −1 s to +2.5 s and −800 to +200 ms relative to target onset). All epochs with a peak pupil diameter exceeding ±2 mm were rejected (Murphy et al., 2011). We extracted two variables of interest: (i) pupil dilation (PD), as the peak value of the maximum dilation after targets presentation, and (ii) pre-stimulus pupil diameter (PrePD), as the mean of 1 s data prior to tone presentation.

2.5 | Statistical analyses

As expected, the nature of our oddball task caused ceiling effects in the correct responses for all conditions (accuracy rate >98%). All trials including either a false alarm or a missed response were removed from subsequent analyses, as well as trials corresponding to RT faster than 150 ms or exceeding 1.96 standard deviations from the mean (number rejected trials: $M = 3.14$, $SD = 1.39$). All valid RT were then log-transformed to the base *e* in order to ensure a normal distribution of the data.

We considered only trials having no missing values at the two main outcomes RT and PD, resulting in 52 trials overall. Importantly, these data points were not collapsed across conditions: *Trial* was included in the analyses as an independent fixed factor, and thus affording a greater reliability and robustness of the findings.

In order to study the effect of tDCS on the behavioural and physiological responses, we performed two linear mixed models (LMM) on RT and on PD as dependent variables. Individual (subject-specific) variation was accounted for by considering *Subjects* as random effect. Fixed effects, repeated within subjects, were specified for *Condition* (2 levels, *real* and *sham*), *Time* (2 levels, *T1* and *T2*) and *Trial* (52 levels), whereas *Order* (2 subgroups, *sham-real* and r*eal-sham*) was considered as a between-subject fixed effect. In addition, the interaction *Condition* × *Time* was assessed. Post hoc comparisons were adjusted with Sidak correction for multiple comparisons. The use of all repeated measures (including the 52 levels of Trial) at each time point allowed to considerably increase the power of the tests on the assessed effects that is, notoriously, affected by the sample size (see, e.g., the degree of freedom—reported in subscript parentheses—of the statistics of each effect reported in Section 3). The use of LMMs allows to manage and use all the data available at each time point in a repeated measure framework, and this, in turn, allows to increase the statistical power of the analysed effects. A post hoc power analysis was performed to provide proofs of reliability and robustness of our LMM results. In detail, once the LMMs were performed, the power of each fixed effect of LMMs was analysed by *powerSim* function of the *simr* package of the R software (R core Team, 2020, http://www.R-project.org/), which provides both the power of the estimated effect and the corresponding 95% confidence interval.

The above LMM was subsequently adjusted for subjective arousal (measured by STAI-Y State score) and for physiological arousal (evaluated by PrePD) in order to assess their effects on tDCS-induced modulation. Akaike information criteria (AIC) was used to select the best fitted models (the lower AIC the better model) and the corresponding predictors.

3 | RESULTS

3.1 | Reaction times

The unadjusted linear mixed model on reaction times (RT) (AIC = −2545.34) revealed no significant effects of the *Order* ($F_{(1,16)} = 0.071$, $p = 0.794$) and *Trial* ($F_{(51,126)} = 1.10$, $p = 0.322$). A significant effect of *Time* ($F_{(1,2999)} = 4.53$, $p = 0.033$) showed that performance significantly improved from *T1* to *T2* sessions, indicating an overall practice effect. In addition to a significant effect of *Condition* ($F_{(1,3040)} = 11.40$, $p = 0.001$), we found a significant *Condition* × *Time* interaction effect ($F_{(1,3014)} = 10.96$, $p = 0.001$), indicating a different trend for real and sham conditions. The post hoc comparison within the factor *Time* revealed a significant performance improvement during *sham* ($p < 0.001$), but not during *real* stimulation ($p = 0.95$). This finding suggests that real tDCS hindered the practice effect that was present in the sham condition.

Next, LMM adjusted for STAI-Y and PrePD were separately performed (see Table 1). We found an overall significant contribution of STAI-Y ($F_{(1,2886)} = 7.95$, $p = 0.005$) and more importantly a significant three-way interaction (*Condition* × *Time* × STAI-Y: $F_{(3,2108)} = 12.53$, $p < 0.001$), indicating that the subjective level of arousal affected the interaction *Condition* × *Time* on RT in different way across time (T1 and T2) and conditions. Specifically, STAI-Y state scores were predictive of the performance variations across tDCS conditions: During sham session, a performance improvement (i.e., a decrease of RT in T2 stimulation) was observed for almost all the levels of arousal, and it visibly diminished at the higher end of the STAI-Y range. Differently, in the real tDCS condition, the improvement of RT reversed in a worsening pattern, with a more substantial performance decline (i.e., an increase of RT in T2 stimulation) as STAI-Y scores increased (see Figure 2 for a graphical display of the *Condition* × *Time* × STAI-Y significant effect).

TABLE 1 The structure and statistics of the models utilized for the dependent variable reaction time

Behavioural performance (RT)				
Models	**AIC**	**Fixed factors**	$F_{(df)}$	***p* value**
(1) Unadjusted model	−2545	*Trial*	$1.10_{(51,126)}$	0.322
		Time[a]	$4.53_{(1,2999)}$	0.033
		Order	$0.07_{(1,15)}$	0.794
		Condition[a]	$11.40_{(1,3040)}$	0.001
		Condition × *Time*[a]	$10.96_{(1,3014)}$	0.001
(2) Adjusted for STAI-Y	−2536	STAI-Y[a]	$7.95_{(1,2886)}$	0.005
		Trial	$1.09_{(51,130)}$	0.334
		Time[a]	$24.83_{(1,2864)}$	<0.001
		Order	$0.11_{(1,16)}$	0.74
		Condition	$1.74_{(1,2868)}$	0.187
		Condition × *Time* × STAI-Y[a]	$12.53_{(3,2108)}$	<0.001
(3) Adjusted for PrePD	−2519	PrePD	$2.80_{(1,2462)}$	0.094
		Trial	$1.02_{(51,124)}$	0.483
		Time	$1.47_{(1,2734)}$	0.225
		Order	$0.10_{(1,16)}$	0.745
		Condition[a]	$8.54_{(1,2903)}$	0.003
		Condition × *Time* × PrePD[a]	$5.40_{(3,1772)}$	0.001

Note: All models include subjects as random factor. Significant fixed factors are displayed with an asterisk.
[a]Significant effect.

After adjusting for PrePD, the effect of *Condition* remained significant ($F_{(1,21903)} = 8.54$, $p = 0.003$), but this was not the case for neither the effect of *Time* nor the physiological predictor ($F_{(1,2734)} = 1.47$, $p = 0.225$; $F_{(1,2462)} = 2.80$, $p = 0.094$, respectively). However, the significant three-way interaction effect (*Condition* × *Time* × PrePD: $F_{(3,1772)} = 5.40$, $p = 0.001$) revealed that the participants' physiological level of arousal affected the RT in a different way across condition and time: Performance improvement across time was consistent in the sham condition, whereas RT became slower as ongoing physiological arousal increased (see Figure 3 for a graphical display of *Condition* × *Time* × PrePD significant effect).

By the post hoc power analysis, a power higher than 0.8 was found for all significant effects of the mixed models above reported, except for the effect of *Time* on reaction times ($F_{(1,2999)} = 4.53$, $p = 0.033$; power = 0.5 [95% CI: 23–65%]).

Overall, based on these data, a different trend emerged from the comparison of the investigated effects (i.e., RT change) between adjusted and unadjusted models (Figure 4). When using our adjusted models, the evident reduced variability in the direction of RT change corroborates the importance of not disregarding discrepancies rooted in interindividual differences, such as in physiological and subjective arousal, but rather include them as predictors along with individual random effects.

3.2 | Pupil dilation

In the unadjusted LMM on pupil dilation (PD), AIC = 857.37, all fixed effects were significant (*Condition*: $F_{(1,2069)} = 10.30$, $p = 0.001$; *Time*: $F_{(1,2102)} = 34.58$, $p < 0.001$; *Trial*: $F_{(51,121)} = 6.06$, $p < 0.001$) except for the factor *Order* ($F_{(1,15)} = 0.59$, $p = 0.45$) and the interaction between *Condition* and *Time* ($F_{(1,2080)} = 2.94$, $p = 0.086$). Importantly, pupil dilation decreased from *T1* ($M = 0.36$; $SE = 0.021$) to *T2* sessions ($M = 0.31$; $SE = 0.021$), indicating a general habituation of the phasic pupillary responses. However, no specific effect of tDCS on PD was revealed.

The adjustment for STAI-Y got slightly worse the model fitting (AIC = 872.86) (see Table 2), though with a significant effect of the predictor

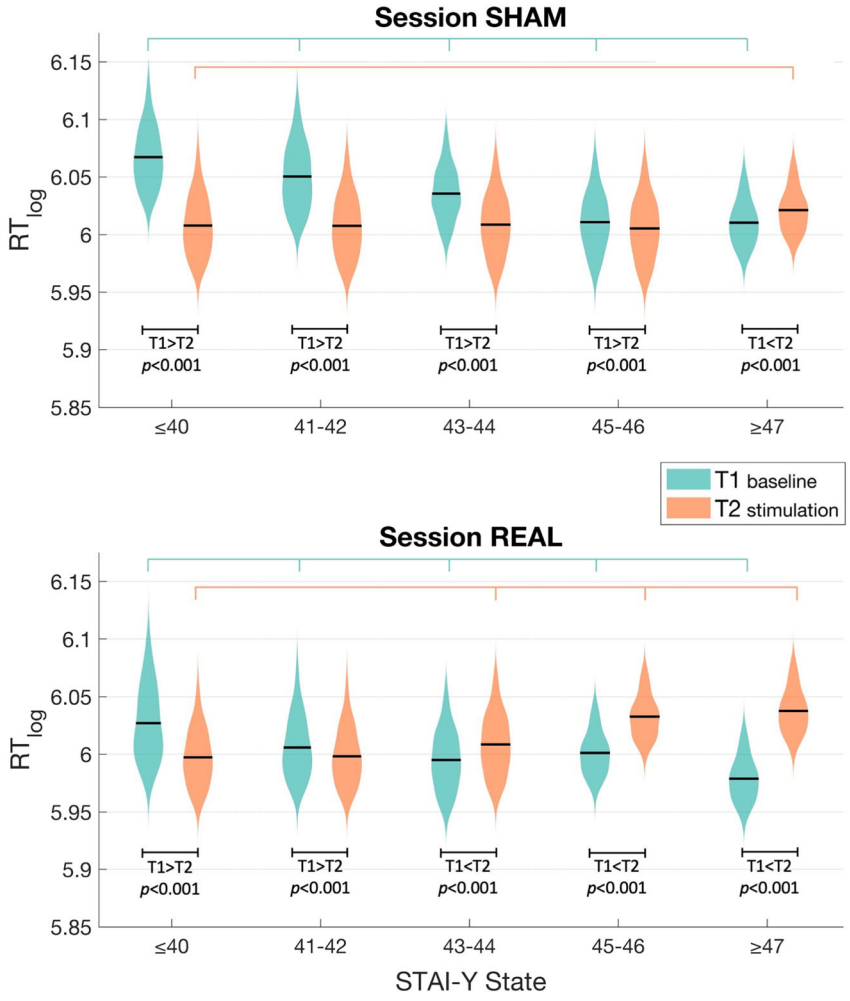

FIGURE 2 Reaction times by subjective arousal, condition and time. Average log-based RT of model fitted values is plotted as a function of STAI-Y scores (binned values), with results from session sham (top panel) and real (bottom panel) in the two time points T1 and T2. Each mean value is marked over the corresponding distribution of the data. Colours light green and orange represent the baseline (T1) and stimulation (T2) task, respectively. The figure aims to provide graphical evidence for the interaction term: Condition × Time × STAI-Y. For this purpose, the continuous variable STAI-Y was categorized in five binned values. In the session *sham*: Baseline RTs decreased as STAI-Y State scores increased (i.e., baseline RTs at ≤40 score were slower than RTs at the other scores, all *p*s < 0.001). No difference in RTs were found for T2 evaluation across the STAI-Y range, except for ≤40 versus ≥47, $p < 0.001$. In the session *real*: Baseline RTs decreased as STAI-Y State scores increased (i.e., baseline RTs at ≤40 score were slower than RTs at the other scores, $p < 0.001$ for all). An increase in RTs was found for T2 evaluation across the STAI-Y range (i.e., T2 RTs at ≤40 score were faster than RTs at other scores, all *p*s < 0.001, except for the 41–42 score)

($F_{(1,1744)} = 17.92$, $p < 0.001$) and a significant interaction *Condition* × *Time* × STAI-Y ($F_{(3,1521)} = 3.01$, $p = 0.029$), suggesting that pupil dilation was also affected by tDCS according to the subjective levels of arousal.

Adjusting for PrePD strongly improved the model fitting (AIC = −658.59), with significant PrePD ($F_{(1,2548)} = 2337.89$, $p < 0.001$) and interaction *Condition* × *Time* × PrePD effects ($F_{(3,1517)} = 12.58$, $p < 0.001$). In detail, during the sham condition, a decrease in pupil dilation consistently occurred throughout the range of PrePD values, whereas during real tDCS, the pupil dilation progressively shifted towards a greater reduction during trials with larger PrePD (see Figure 5 for a graphical display of *Condition* × *Time* × PrePD significant effect).

By the post hoc power analysis, a power higher than 0.8 was found for all significant effects of the mixed models, except for the interaction effect *Condition* × *Time* × STAI-Y on pupil dilation ($F_{(3,1521)} = 3.01$, $p = 0.029$; power 0.6 [95% CI: 36–81]).

4 | DISCUSSION

In the present study, we addressed the question of whether variable effects of a single tDCS session could be dependent on the degree of arousal experienced before (i.e., subjective report of anxiety at baseline, STAI-Y) and during (i.e., physiological tonic LC activity, PrePD) the experiment. We found that subjective and physiological levels of arousal significantly accounted for the variation of reaction times across two experimental sessions. Real tDCS appeared to hinder the practice effect observed during the sham condition, with a trend becoming especially evident at higher levels of arousal. As for pupil dilation, its modulations matched with the behavioural results, with a more reduced pupillary response emerging during real tDCS as arousal levels increased.

These results shed light on one relevant factor, which may account for the paucity of consistency across tDCS effects: Arousal appears to be predictive of the

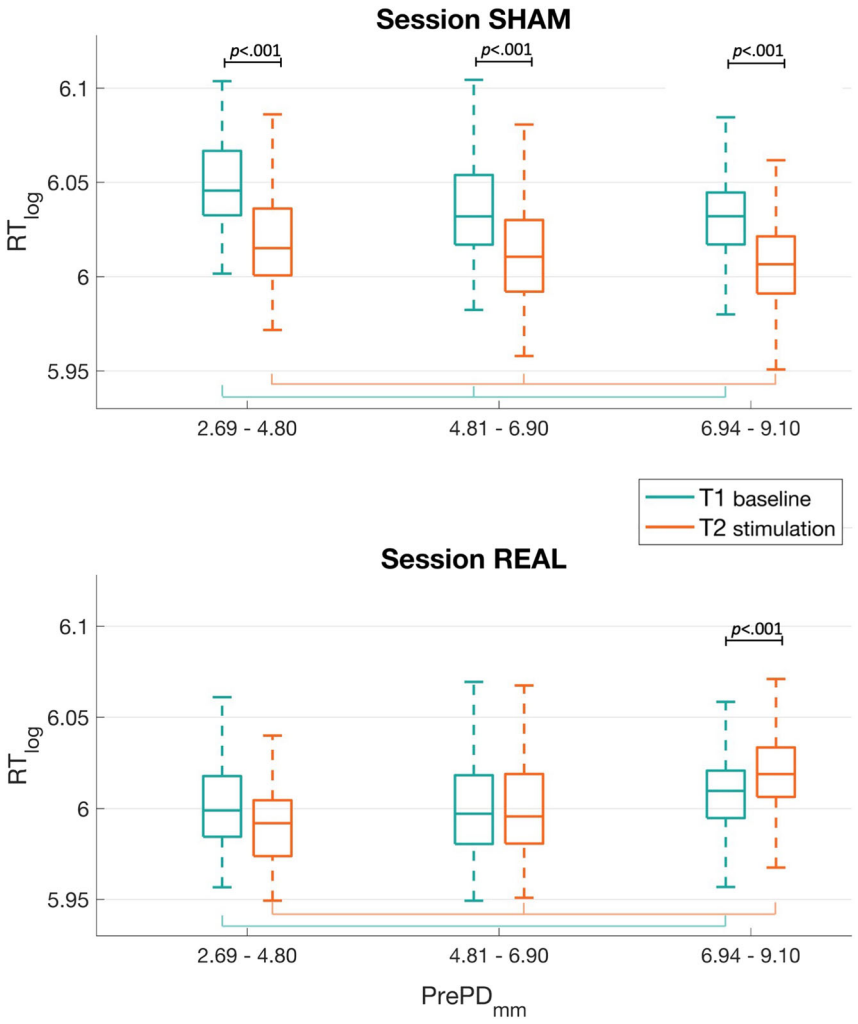

FIGURE 3 Reaction times by physiological arousal, condition and time. On each box, the interquartile range, the whiskers and the median of predicted log-based RT are represented for three linearly interspaced bins of pre-target pupil diameter, with results from session sham (top panel) and real (bottom panel) in the two time points T1 and T2. Colours light green and orange represent the baseline (T1) and stimulation (T2) task, respectively. The figure aims to provide graphical evidence for the interaction term: Condition × Time × PrePD. For this purpose, the continuous variable PrePD was categorized in three binned values. In the session *sham*: A decrease in RTs was found for the T1 evaluation across the PrePD bins (i.e., T1 RTs at first bin were *slower* than RTs at other bins, all *p*s < 0.001). A decrease in RTs was found for the T2 evaluation across the PrePD bins (i.e., T2 RTs at first bin were *slower* than RTs at other bins, all *p*s < 0.05). In the session *real*: for the T1 evaluation RTs increased only comparing the first bin to the third bin ($p < 0.05$). An increase in RTs was found for T2 evaluation across all the PrePD bins (i.e., T2 RTs at first bin were *faster* than RTs at other bins, all *p*s < 0.01)

modulations induced by tDCS on task performance. A number of studies, which reported a considerable inter- and intra-individual variability in response to tDCS protocols, investigated the impact of demographic characteristics (e.g., age and gender), cortical architecture variations or physiological measures specific to the targeted areas (e.g., levels of excitability of the primary motor cortex), yet without considering general measures of activation comparable to arousal (Filmer, Ehrhardt, Shaw, et al., 2019; Katz et al., 2017; López-Alonso et al., 2015; Ridding & Ziemann, 2010; van de Ruit & Grey, 2019; Wiethoff et al., 2014). Here, we collected ratings on the subjective level of anxiety (i.e., STAI-Y State) before each experimental session. Due to the close relation between the LC activity and the perceived anxiety (Eysenck, 1963; Mizuki et al., 1997; Robbins & Everitt, 1995), we used it as a fixed measure of arousal. Pre-target pupil diameter was instead used as a dynamic proxy of arousal, allowing us to track its ongoing fluctuations (Murphy et al., 2011; Unsworth et al., 2018; Van Den Brink et al., 2016). We confirmed that pupil dilation values were negatively related with pre-target pupil diameter across all conditions, as frequently reported in the literature (De Gee et al., 2014; Hong et al., 2014; Murphy et al., 2011). Remarkably, despite the limited sample size, the large number of repeated measures properly used in a linear mixed model fashion allowed us to obtain an adequate power and thus robustness of our results.

When our measures of arousal were accounted for by statistical analyses, a clearer picture emerged, indicating that the effects induced by tDCS on the behavioural responses interacted with both subjective and physiological levels of arousal. Participants speeded up their responses when they completed the task for the second time in the sham session. This practice effect emerged somewhat independently of the ongoing physiological levels of arousal, although a more pronounced improvement appeared with lower levels of subjective arousal. During the application of real tDCS, however, performance ceased to improve more dramatically and, in fact, reversed with the exception of trials characterized by smaller pre-target pupil diameter and participants with a

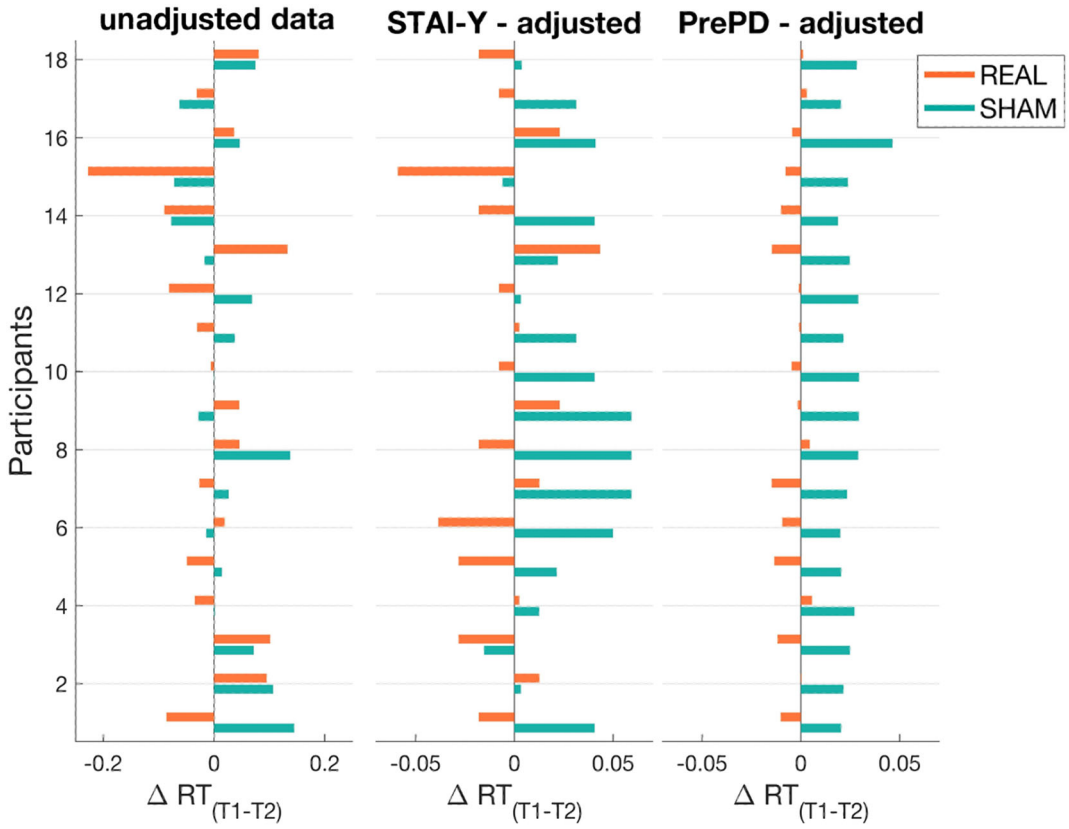

FIGURE 4 Subject variability of reaction time change. Average log-based RT differences between the baseline (T1) and stimulation (T2) tasks are plotted on the vertical axis for each participant, separately for session sham (green bars) and real (orange bars). Each different bar plot is used to represent mean differences from the unadjusted (left panel) and the adjusted models using STAI-Y (middle panel) and PrePD (right panel) predictors. Negative and positive values on the horizontal axis indicate slower and faster performance, respectively

lower score at the STAI-Y questionnaire. A negative or null behavioural outcome of anodal tDCS is not uncommon in the literature and learning impairments have been reported in a host of different tDCS studies involving specific learning outcomes, such as unimproved working memory for recognition or implicit categorization, blocked consolidation of visual perception and inhibited motor learning (Ambrus et al., 2011; Berryhill et al., 2010; Bortoletto et al., 2015; Fertonani et al., 2011; Peters et al., 2013; Verhage et al., 2017). We chose response speed as behavioural measure, given that its intrinsic low sensitivity heavily relies on prior levels of fatigue and general activation (Welford, 1980). The interpretation of our behavioural results could be partially consistent with an inverted U-shape curve between task performance and arousal. According to this relationship, performance decline would occur when arousal levels are either too high or too low (Yerkes & Dodson, 1908). However, because none of the participants reported sleep deprivation or otherwise drowsiness-related conditions, we can assume that the lower values of our predictors effectively corresponded to moderate and not low levels of arousal. With this in mind, the finding that facilitatory effects are principally associated with a moderate level of cortical excitation also seems to support the proposed cellular mechanism for a cortical excitation-inhibition balance (Krause et al., 2013). On these grounds, tDCS exogenous modulation would negatively impact on the normal cortical functioning whenever the levels of endogenous neural activity increase to the extent of a dysfunctional neuronal gain, with spontaneous task disengagement causing slower responses (Smallwood et al., 2004). A direct consequence of this mechanism would be the inhibition of task learning effects, unless the endogenous system is sufficiently inactive, as in low arousal trials. The latter scenario would provide an argument for when single session tDCS is found to improve task performance in the face of variable but otherwise moderate and well-balanced arousal levels. Moreover, the understanding that an unbalanced combination of endogenous and exogenous excitability-increase events may lead to negative effects is also coherent with frameworks on brain activity-dependent plasticity and on signal-to-noise ratio mechanisms (Bortoletto et al., 2015; Fertonani & Miniussi, 2017).

TABLE 2 The structure and statistics of the models utilized for the dependent variable pupil dilation

Pupil dilatation (PD)				
Models	**AIC**	**Fixed factors**	$F_{(df)}$	***p* value**
(1) Unadjusted model	857	*Trial*[a]	$6.06_{(51,121)}$	<0.001
		Time[a]	$34.58_{(1,2102)}$	<0.001
		Order	$0.59_{(1,15)}$	0.451
		Condition[a]	$10.30_{(1,2069)}$	0.001
		Condition × *Time*	$2.94_{(1,2080)}$	0.086
(2) Adjusted for STAI-Y	872	STAI-Y[a]	$17.92_{(1,1744)}$	<0.001
		Trial[a]	$6.13_{(51,118)}$	<0.001
		Time[a]	$3.98_{(1,2069)}$	0.046
		Order	$1.44_{(1,16)}$	0.247
		Condition	$0.06_{(1,2009)}$	0.807
		Condition × *Time* × STAI-Y[a]	$3.01_{(3,1521)}$	0.029
(3) Adjusted for PrePD	−686	PrePD[a]	$2337.89_{(1,2548)}$	<0.001
		Trial[a]	$2.29_{(51,119)}$	<0.001
		Time[a]	$19.05_{(1,2683)}$	<0.001
		Order[a]	$7.84_{(1,15)}$	0.013
		Condition[a]	$7.85_{(1,2158)}$	<0.001
		Condition × *Time* × PrePD[a]	$12.58_{(3,1517)}$	<0.001

Note: All models include subjects as random factor. Significant fixed factors are displayed with an asterisk.
[a]Significant effect.

The above interpretations are accompanied by results on pupil dilation, which represents a physiological response to relevant stimuli. When the ongoing levels of arousal were considered in the analyses, a specific effect of tDCS on pupil dilatation was revealed. An overall reduction of pupil dilation occurred when participants completed the task for the second time, consistently with a physiological habituation effect that paralleled the practice effect seen in the behavioural results (Rankin et al., 2009; Thompson & Spencer, 1966). In particular, pupil dilation evenly decreased for the entire range of arousal in the sham session, but different variations emerged during the application of real tDCS: As compared with the lower end of the arousal range, a more pronounced reduction in pupil dilation was observed in trials associated with higher physiological arousal. These, in fact, fell towards the same right end of the scale as the trials of unimproved response times during real tDCS. Therefore, habituation of a phasic response may not necessarily match the outcome direction of the behavioural improvement (Mackworth, 1968). Pupil dilations primarily reflect the timely increase of neural gain control, which translates into a system's responsivity amplification, and as such can be ascribed in the aforementioned inverted-U curve (Aston-Jones & Cohen, 2005; Hong et al., 2014; Murphy et al., 2011). The implication would be that the additive effect of an exogenous neuromodulation would, on the one hand, contrast the natural habituation effect on pupil dilation occurring below the intermediate range of tonic arousal and, on the other hand, accentuate task disengagement at higher levels of tonic arousal, hence a greater reduction in phasic response. An analogous explanation was put forward in a recent tDCS work showing a reduction of pupil dilation—but no behavioural effects—during a Go-NoGo task, whereby it was argued that an offline tDCS enhancement of neuronal membrane potential could hinder or replace the endogenous gain control mechanisms of locus coeruleus (Adelhöfer et al., 2019): When participants were asked to withhold their responses, a correlation between the theta band inhibitory activity and pupil dilation was present during the sham stimulation, but not during real tDCS condition.

Furthermore, outside the tDCS literature, phasic pupillary responses were found to be reduced whenever participants' attention was not directed to the task, such as during episodes of mind wandering (Jubera-García et al., 2019; Mittner et al., 2014; Smallwood et al., 2011; Unsworth & Robison, 2016). Indeed, recent empirical and theoretical formulations of mind wandering have proposed that the locus coeruleus–norepinephrine system is tightly linked to different internally driven cognitive states, that

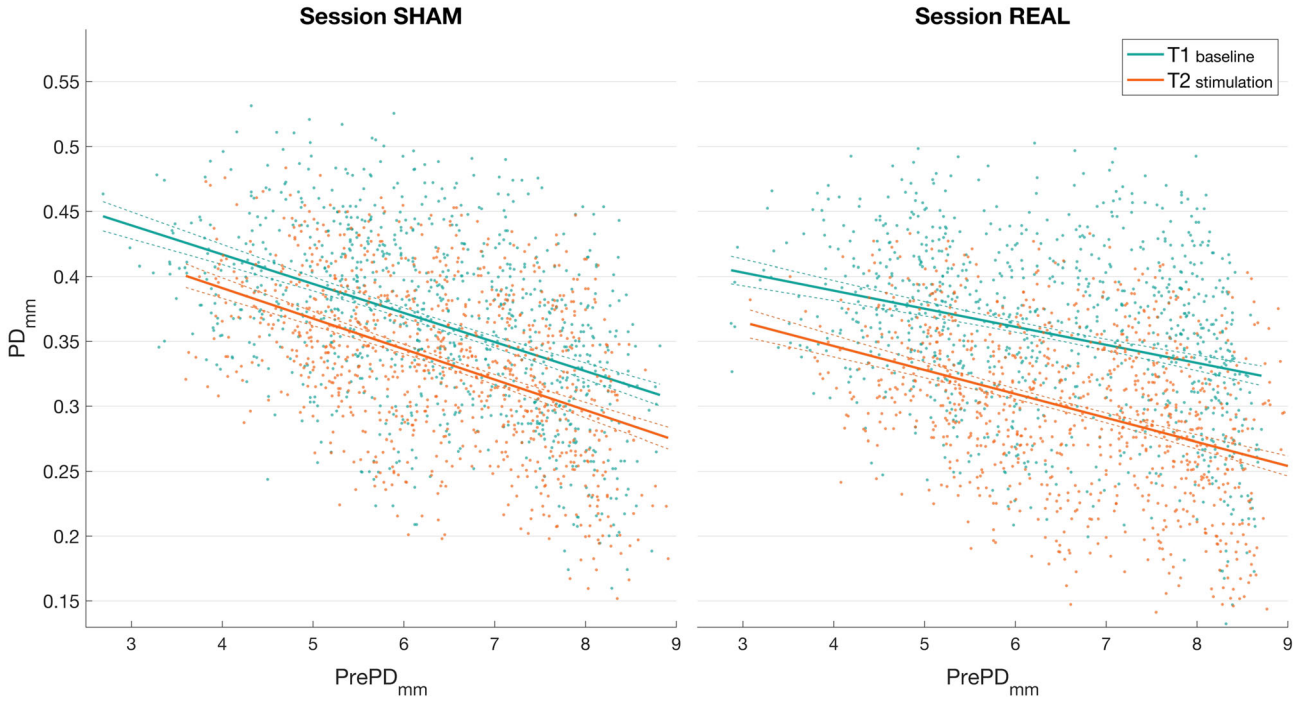

FIGURE 5 Pupil dilations explained by physiological arousal. Model fitted PD values are plotted against pre-target pupil diameter, with results from session sham (left panel) and real (right panel). Light green and orange best-fitting lines describe the trend of pupil dilation data points over pre-target pupil diameter respectively for the baseline (T1) and stimulation (T2) task. Dashed lines represent prediction functional bounds, that is, the uncertainty of predicting the fitted lines. The figure aims to provide a graphical evidence for the interaction term: Condition × Time × PrePD. In this case, the continuous variable PrePD needed not be categorized in bins because of the trial-by-trial correspondence with the PD values. Hence, we fitted a straight line as a way to better visualize the significant effect

is, on- and off-task states with various degrees of deliberate control (Christoff et al., 2016; Mittner et al., 2016). In this respect, the possibility of a direct and focally targeted tDCS modulation of mind wandering has been recently debated with uncertain conclusions (Chaieb et al., 2019). Based on this knowledge, it is not unlikely, although only hypothetical, that our tDCS effects would also be partly dependent on the arousal-mediated propensity of mind wandering activity during the task. Alternatively, mindfulness disposition, which is often considered to be in anticorrelation with mind-wandering, might lend itself to an analogous interpretation (Seli et al., 2016). Interestingly, it was recently found that tDCS decreased performance in a sustained attention task only in participants with high and not low dispositional mindfulness (Molina et al., 2020). This finding fits well with the idea of different internal states whose degree of intentionality and control always reflect the LC-driven transitions between explorative and exploitative mental operations (Mittner et al., 2016).

In summary, our data highlight a critical factor that could explain the negative or null effects of a common tDCS application. We are aware that these are preliminary results, and their interpretation may not directly generalize to all tDCS studies until further support is provided.

As arousal is considered a base layer for many high-order cognitive functions (Berridge & Waterhouse, 2003), our rationale behind the stimulation set-up did not include the targeting of a precise region of interest. Therefore, this approach differs from what is commonly used in several tDCS studies where the expectation is to define and/or rely upon topographical maps that support brain functions. The lack of an active control condition to exclude montage-specific effects may be considered a limitation of this study, and future research might find appropriate to investigate how and if different electrode arrangements and the involvement of specific functional areas can affect the interactive influence of arousal. On the one hand, a bold hypothesis would be that, regardless of the stimulation site, online tDCS affects the cortical interaction with endogenous modulations e.g., as measured by a behavioural index of phasic arousal (Luna et al., 2020). Hypothetically, a similar effect could be due to different cortical regions embedded in the same long-range specialized network that equally subserve a given cognitive task; alternatively, or in addition to this, we posit that the neural underpinning of arousal—for example, putative widespread noradrenergic projections in the brain—could potentially make the interaction with the induced cortical excitability irrespective of the spatial

localization. On the other hand, though, the direction of the present results may have well depended on the specific interplay between tDCS montage, stimulation site and task. For example, it is possible that for most participants the current flowing through the anode and the cathode affected the orbitofrontal cortex, which is a key area in decision-making, and might have also played a crucial role in our task. The focus of future research should employ high-definition montages as well as carefully considering electric field intensity, focality and direction. Furthermore, the reciprocal interactions between the arousal system's neurotransmitters (e.g., noradrenaline and dopamine) should also be investigated whenever the stimulation site comprises parts of common projections (e.g., prefrontal regions, Briand et al., 2007).

5 | CONCLUSION

The large differences that we found in arousal levels stimulate reflection on what may mask the desired effects in the varied and still growing landscape of neurostimulation studies, which often fail to incorporate, but simply acknowledge, the crucial aspect of individual state-dependent variables (Lanina et al., 2018; Penton et al., 2018; Talsma et al., 2017; Willis et al., 2019). The importance of brain state is not a novel idea in the literature on non-invasive brain stimulation. The ongoing or basal levels of activation, included in the concept of "state-dependency," have been extensively reported to impact the effects of transcranial magnetic stimulation (TMS) (Silvanto et al., 2008) as well as tDCS (Antal et al., 2007). Considering the mechanisms of action of tDCS, which modulates excitability of neurons by hyperpolarizing or depolarizing their membrane potential (Liebetanz et al., 2002; Nitsche et al., 2003), tDCS effects might be even more sensitive to the arousal levels than TMS. In a similar vein, these considerations might be applicable to any kind of current stimulation modality.

Future tDCS studies might also consider useful to have both dynamic and fixed measures of arousal as an accurate way to monitor its impact on the final outcome. If successful, these achievements would be of great help also in assessing the degree of effectiveness with which tDCS protocols are being utilized to treat or ameliorate clinical conditions.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

AUTHOR CONTRIBUTIONS

M.E. was responsible for the study concept and design, data acquisition and analyses and data interpretation and wrote the original draft. C.Fe. was responsible for the data analyses, data interpretation and writing, review and editing. C.Fr. was responsible for the study concept and design, data acquisition and data interpretation. C.M. was responsible for the data interpretation and writing, review and editing. D.B. was responsible for the study concept and design, data interpretation, writing, review and editing and supervision.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

The database containing the raw data used in this study is available at data.mendeley.com, https://doi.org/10.17632/v9kt5v8p2b.1

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