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Research paper

Combined EEG-tDCS approach in resting state to reduce comorbid anxiety and depressive symptoms in affective disorders: A sham-controlled pilot study

Tania A. Couto [a,b], Fei Gao [a,c], Davis C. Lak [d,e], Zhen Yuan [a,*]

[a] Faculty of Health Sciences. Centre for Cognitive and Brain Sciences. University of Macau, Taipa 999078, Macau, Special Administrative region of China
[b] Brain, Language and Computation Laboratory. University Research Facility in Behavioural and Systems Neuroscience. The Hong Kong Polytechnic University, Hong Kong Special Administrative Region of China
[c] Fudan University, Shanghai, China
[d] Alpha Positive Limited, Central, 999077, Hong Kong Special Administrative Region of China
[e] Department of Psychology. The University of Hong Kong, Hong Kong Special Administrative Region of China

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ABSTRACT

Continuous challenges have been imposed on mental health science by Anxiety and Depression disorders as the most prevalent and debilitating psychiatric conditions worldwide. Pharmacologic and cognitive behavioral therapies, either alone or in combination, have been considered as the first-line therapies, however, resistant symptomatology is prevalent in comorbid conditions with symptoms remaining after interventions. The demand for new therapeutic solutions has given space to the development of non-invasive brain stimulation techniques (NIBS), and the transmagnetic direct current stimulation (tDCS) has been reported as a safe and well-tolerated technique for the treatment of several mental health conditions, including Anxiety and Depression disorders. Relying on quantitative electroencephalography(qEEG)- tDCS approach, the current study aims to inspect the effect of tDCS intervention on patients who suffer from anxiety-depression comorbidity, in particular, the impact of tDCS intervention on qEEG spectral power activity and resting-state connectivity organization during eyes closed and eyes open protocols. QEEG data were acquired from eight patients suffering from moderate to severe anxiety-depression comorbid symptoms along with poor coping skills to manage stress and negative affect. Twelve control subjects allocated in the control group exhibiting low to moderate symptoms in both anxiety and depression conditions went also through the qEEG data acquisition. In addition, a sham-controlled study was conducted, and the patient group went through resting-state qEEG-tDCS neuromodulation once a week for ten weeks. Various-stage qEEG recordings were performed to inspect the efficacy of tDCS treatment during the modulation of brain regions involved in the regulation of affective responses. Our results demonstrated that after tDCS neuromodulation, the patients' groups exhibited decreased absolute power abnormalities over the left anterior cingulate cortex and reduced abnormal activity in the alpha band over posterior regions; improved functional connectivity indexes; decreased anxiety and depressive scores while positive affect score was improved. Besides the promising improvements, our study did not find a significant tDCS effect on perceived stress and negative affect scores. Consistently, significant differences in absolute spectral power over the left anterior cingulate cortex were detected among the patient group, as compared to the controls, as expected. Therefore, our study offers preliminary data to understand the neuroplasticity changes that potentially result from the manipulation of cortical excitability during affective regulation protocols followed by the consequent decrease of comorbid anxiety and depressive symptomatology. The pilot study was followed by prospective registration with ChiCTR2200062142.

* Corresponding author.
E-mail address: zhenyuan@umac.mo (Z. Yuan).

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1. Introduction

Humans react differently to the same emotional experiences according to their assessment of a situation and their mindset, values, and subjective preferences (Lazarus, 1991), (Vergallito et al., 2018a). When exposed to highly stressful conditions, humans are not always equipped with the appropriate coping skills to manage affective responses effectively. As a result of this exposure, exaggerated emotional responses and inefficient regulation strategies lead to a dysfunctional cycle of negative affect and prolonged fear responses among psychological sufferings. It is well established that both responses co-occur in the development of affective disorders such as generalized anxiety disorder (GAD) and major depressive disorder (MDD) and a significant number of studies have successfully addressed these symptoms (Bikson and Rahman, 2013). However, there is a lack of studies confirming the effect of repeated sessions of transmagnetic direct current stimulation (tDCS) in anxiety disorders, and there is a substantial gap of electroencephalography evidence in the literature supporting the improvement in scores in relation to both depression and anxiety conditions. In addition, it remains unclear how the tDCS effect impacts the functional connectivity of regulatory dynamics.

In order to conceptualize the tDCS mechanism of action, Bickson and Rahman (Bikson and Rahman, 2013) propose a solid framework which relies on three models: (i) anatomical specificity; (ii) activity-selective; and (iii) input-bias mechanisms. The first model — anatomical specificity – refers to the neuromodulation of a targeted brain region by delivering stimulation current to a targeted area (for example, the targeted main regions which represent a specific cortical area involved in a task or pathology). The second model relies on activity-selectivity which assumes that tDCS will preferentially modulate specific forms of ongoing activity. In more detail, direct current stimulation (DCS) may facilitate long-term potentiation and membrane polarization but only those activated during DCS (by task or experimental stimulation) would benefit from this facilitation. The third and last model — input selectivity — does not presuppose co-activation (as described in the second model) and does not require anatomical targeting of current flow, which assumes that the neuronal network is predisposed to serve at least two functions or operate in at least two states. In more detail, tDCS may switch the network from one function/state to another, which emphasizes that one process may be enhanced at the cost of enhancing another. According to the authors, this concept can be extended to disease states such as anxiety and schizophrenia (Bikson and Rahman, 2013), (Grace, 2000), and depression (Loo et al., 2012), or to two functions that interestingly compete between them to be modulated (Levy, 2004).

An influential model proposed by Phillips et al (Phillips et al., 2003). offers relevant insights to understand how neuromodulation might help to coordinate the efforts of the neural systems during regulatory dynamics. The conceptualization highlights the dorsal and ventral systems as two central systems that play a pivotal role in a reciprocal cycle during two different stages: from the production of an affective state to the regulation of emotional responses. The ventral systems are primarily responsible for the emotional significance of a stimulus, the production of an affective state, and the autonomic response regulation, while the dorsal system is responsible for recruiting the executive functions (e.g., selective attention and planning), regulating affective states, and consequent behaviour. Enlighted by this framework, recent evidence (Vergallito et al., 2018b), (Van 't et al., 2016) confirms that these target circuits are involved in the modulation of key affective responses for evolutionary adaptation, such as fear and negative emotionality. A doctoral work targeting fear and threat responses (Ironside et al., 2016) adds that the dorsal lateral prefrontal cortex seems to inhibit the amygdala response to threat in humans, providing support to the evidence that the vigilance to threat is mainly coordinated by the DLPFC—amygdala hub. According to the author (Ironside et al., 2016), this hub might represent a key neuro mechanism for the efficacy of tDCS in the treatment of depression and anxiety disorders.

In line with previous evidence, the existing task-related findings in affective regulation highlight the successful extinction of conditioned fear related to the top-down modulation of the ventromedial prefrontal cortex (vmPFC), which is also moderated by the amygdala. In more detail, the connectivity hub — amygdala—vmPFC – seems to contribute to the extinction process while the activation of vmPFC has been reported to be an effective strategy not only to reduce fear but also to enhance the mnesic processes responsible for its extinction (Van 't et al., 2016). Regarding negative emotion modulation, the evidence suggests that the chronic inability to suppress negative emotions is correlated with structural and functional brain abnormalities (Vergallito et al., 2018b), (B. AL, S. Saxena, M. MA, F. LA, H. ML, and B. LR, 2001). For example, in comparison with healthy controls, patients with depression showed decreased neural activity over the prefrontal area, increased activity in the anterior cingulate cortex and amygdala, and reduced volumes of the frontal lobe, hippocampus, and basal ganglia.

Additionally, tDCS studies at rest targeting emotional dysregulation in depression propose the neural system hypothesis, which conceptualizes depression as a condition related to dysfunction in the several cortical and subcortical areas, especially the dorsomedial and ventral areas of the prefrontal cortex (PFC). In line with this hypothesis, significant improvements were confirmed by depressive scores on the Hamilton Rating Scale for Depression (HRSD) in several tDCS studies presented in a systematic review (Ferrucci; Fregni et al., 2006; Ho et al., 2015; Kekic et al., 2016; Knotkova, 2012; Player; Valiengo, 2013; Ferrucci et al., 2009; Dell and Osso,); however, these studies do not offer a neuroimaging or electroencephalography approach to illustrate the effect of tDCS on neural signatures. From this set of studies, only one pilot study provided computational modelling to illustrate the effect of direct current in one healthy patient, and did not offer any control condition (Ho et al., 2014). Also, it remains unclear as to what inclusion criteria were employed for some studies, since they do not inform DSM diagnostics for Major Depressive Disorder (MDD) (Fregni et al., 2006, 2006; Knotkova et al., 2012). Finally, studies treat anxiety as an extension of MDD and do not target the regions involved in the maintenance of core responses in anxiety, such as threat and fear. Therefore, the lack of specificity of the protocols might not entirely address the complexity of the comorbid states, since it does not distinguish fear and threat from negative emotions, and it might require different protocols to perform a successful regulation.

Concerning the efficacy of tDCS studies in generalized anxiety disorder (GAD), tDCS has emerged as a promising solution to address one of the most prevalent and debilitating psychiatric conditions worldwide (Stein et al., 2020a; Stein et al., 2020b). Previous studies rely on the hemispheric imbalance hypothesis which proposes the hypoactivity of DLPFC to be associated with negative emotion judgement and hyperactivity on the right-hand side, linked to attention modulation. Neuromodulation techniques such as TMS and tDCS have been applied in clinical settings to treat anxiety-related disorders, traditionally approached by pharmacology and cognitive behavioral approaches. The described imbalance found in anxiety patients may be treated using the bicephalic montage and preclinical and clinical studies report on behavioural and psychological changes, followed by the alterations in cortical—spinal excitability parameters. While tDCS studies in depression have shown promising efficacy, there are a reduced number of studies investigating anxiety disorder such as GAD (de Lima et al., 2019). The first systematic review (Stein et al., 2020a) revealed the need for high-quality studies in this field highlighting the importance of repeated sessions (at least 10) and the long-term benefits of tDCS in GAD patients. The second systematic review investigating the effects of non-invasive brain stimulation (NIBS) in GAD (Sagliano et al., 2019) reports one single case study of tDCS (Shiozawa et al., 2014), and no sham-controlled tDCS study has been found. Finally, the only randomized controlled trial conducted so far confirms the role of the right DLPFC for the treatment of anxiety in a middle-sized sample (Stein et al., 2020a).

Lastly, when comorbid symptoms are considered, a study using a blind design (Movahed et al., 2018), confirmed the effect of tDCS therapy in both depression and anxiety, combined with pharmacology. A case report also attempted to apply fNIRS—tDCS to both anxiety and depressive conditions and confirmed the efficacy of the therapy, highlighting improvement in social behaviour and sleep behaviour (Wu et al., 2022).

Although the previous neuromodulation studies and the influential framework have paved the way to further understand emotional processing and the regulatory dynamics in healthy participants, the current evidence does not explain how neural plasticity induction within ventral—dorsal systems might be translated into reductions in symptomatology, due to the absence of clinical data. Relying on the existing background in clinical studies, there is a lack of studies confirming the effect of repeated sessions of tDCS in GAD, and there is a substantial gap of electroencephalography evidence in both conditions. The majority of tDCS studies rely on score improvement, which gives room to the combined EEG-tDCS approach presented in the current study. As a complementary assessment strategy, qEEG has been extensively used to inspect the neural correlates of emotional responses; however, as far as we know, a combined qEEG-tDCS methodological strategy has never been carried out to: i) inspect the abnormal power activity involved in the regulatory dynamics after tDCS neuromodulation, and ii) capture the functional connectivity through different indexes (amplitude asymmetry, coherence and phase lag) to measure potential neuroplasticity changes.

Collectively, a qEEG—tDCS combined strategy supports our motivation to conduct an intervention in affective regulation to investigate neural plasticity over time. To investigate this, the study aimed to use qEEG—tDCS to inspect the therapeutic effect of tDCS intervention in patients who suffer from dysfunctional regulation in anxiety—depression comorbidity. In particular, the impact of tDCS intervention on qEEG spectral power activity and resting-state connectivity indexes during eyes-closed and eyes-open protocols was examined. It is expected that the integration of both protocols of negative emotion and fear modulation can significantly reduce the abnormal spectral power activity and enhance connectivity efficiency, while ameliorating the respective comorbid symptoms. Further, the inspection of connectivity indexes, combined with absolute spectral power indexes, can better explain the failure in regulatory dynamics, while the integration of both protocols can provide a reliable approach to target the comorbidity in affective disorders.

2. Materials and methods

2.1. Participants and clinical diagnosis

The study recruited 20 participants in the qEEG study (mean age: 34.4 years, SD = 9.4), eight of whom participated in the qEEG-guided tDCS intervention and 12 served as a control group. The inclusion criteria includes a history of anxiety and depressive symptoms that caused significant distress or impairment in functioning, right-handedness (except two left-handed patients), corrected-to-normal vision, and no history of neurological disorders. The exclusion criteria were the presence of substance use, general medical conditions, and symptoms that are better explained by bereavement. All participants were required to complete four self-report measures to assess anxiety (BAI) (Bardhoshi et al., 2016), depression (BDI) (Beck and Steer, 1988), positive and negative affect (PANAS) (Watson, 1988), and perceived stress scale (PSS) (S. Publications, 1983). The eight patients were then diagnosed with moderate to severe symptoms by a licensed clinical psychologist, and confirmed by a psychiatrist. Of the 20 participants, eight patients received the tDCS intervention. Specifically, the clinical group scored moderate to severe symptoms in BAI and BDI clinical inventories while the control group scored low to moderate symptoms. The participants were recruited through social media and mental health support groups, while data collection and interventions were conducted in a local mental health clinic. The first recruitment was conducted on a self-selection and referral basis, whereas the second stage confirmed the eligibility based on inclusion and exclusion criteria. Before the formal test, the participants received instructions about the experimental procedure and signed written informed consent for each experiment according to the Declaration of Helsinki. The participants received an incentive of HKD100 coupons per session.

The University of Macau's ethical committee approved the protocol for the present study (BSERE21- APP003_ICI/ BSERE21-APP025-ICI) and the pilot study was followed by the Clinical Trial Registration (ChiCTR2200062142) compliant with the World Health Organization (WHO ICTRP - International Clinical Trial Registration Platform); and the Non- Pharmacological CONSORT. The demographic data are provided in Table 1.

2.1.1. Paradigm and Procedures for qEEG-guided tDCS

Resting-state qEEG (Cognionics, Inc) data were collected from 20 participants based on two experimental conditions: eyes-open and eyes-closed. For the first condition (before tDCS intervention), the participants were instructed to perform a resting-state lasting for 3 minutes. They were required to sit quietly with their eyes open, avoid movements, relax their minds, and fixate on one single point. For the eye-closed condition, the participants were asked to close their eyes, rest their minds, and avoid body movements over the three minutes of EEG recordings. Only eight patients received three-session EEG recordings. The first session represented the "BEFORE tDCS" qEEG acquisition. The data for the second session were collected one month later, which denoted the "SHAM" case to examine the symptoms' consistency without tDCS intervention. The qEEG data for the third session were collected "AFTER tDCS" intervention. In the second session of the qEEG data acquisition and clinical ratings, the clinical group received 10-session tDCS once a week for 10 weeks in light of two protocols including the negative emotion modulation and fear extinction. The intervention was performed using a battery-powered stimulator (Activadose, Caputron, United States) with two approximately 4 × 4 cm rubber electrodes coated with electrode conductive gel and saline water. A schematic paradigm is provided in Fig. 2. After the third and last qEEG data collection, the results were discussed in detail with each participant. In addition, we provided a set of cognitive behavioural therapy strategies combined with the approach from the second and third generations to work on the maintenance of the improvements. The same follow-up procedure was implemented with the control group and adjusted according to the severity of the symptoms; however, only the clinical group was submitted to a monthly follow-up scheme during three months starting from the end of the qEEG-guided tDCS study.

2.1.2. Negative emotion modulation

Regarding negative emotion modulation, qEEG-guided tDCS was applied over the rVLPFC (anode) and respective contralateral area (cathode). To stimulate the rVLPFC, the anode was placed over F6 based on the Montreal Neurological Institute (MNI) coordinates retrieved from the international 10–20 system for electroencephalography (EEG) electrode placement (see Fig. 3a). A constant current of 1.5 mA intensity was applied for 20 minutes while participants were asked to sit comfortably and relax: "Close your eyes, relax your mind, and do not move your body."

2.1.3. Fear extinction

We aimed to achieve an intense stimulation over the vmPFC and anterior cingulate cortex, which were recognized to relate to the functions of fear and risk. We calculated the distance of these electrode positions based on the 10–20 system to implement electrode montage (i.e., AF3 for the anode and contralateral mastoid for the cathode; see Fig. 3b). The fear extinction stimulation protocol lasted 10 minutes at a constant level of 2.0 mA. The tDCS ramp-up was conducted gradually to

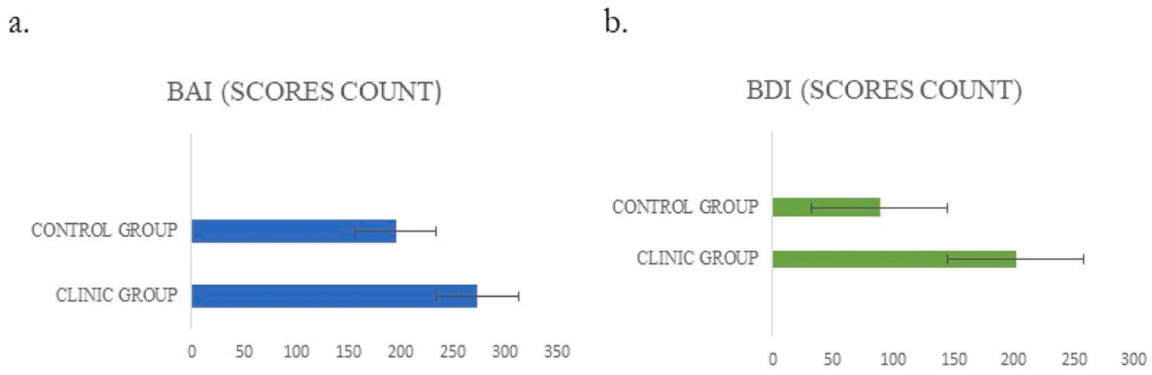

Fig. 1. Group allocation and inclusion/exclusion criteria. The figure a) illustrates the inclusion criteria for clinical group with scores ranging from borderline to severe (21–47 scores count), and exclusion criteria for control group with scores ranging from low to moderate (4–28.5), obtained in Beck Anxiety Inventory (BAI). The figure b) illustrates the inclusion criteria for clinical group with scores ranging from mild to severe (14.5–41 scores count), and exclusion criteria for control group with scores ranging from normal ("ups and downs considered normal") to moderate level (6–21 scores count), obtained in Beck Depression Inventory (BDI). Two participants scoring mild in BDI qualified for clinical group due to the high negative affect (from one to two standard deviation higher than the mean score) combined with poor coping skills to manage anxiety and stress levels.

Table 1
Demographic information of the participants.

	all participants (n=20)	**clinical group (n=8)**	**control group/ qEEG (n=12)**	**control group/ BAI scores (n=12)**	**control group/ BDI scores (n=7)**
Age (M[a], SD[b])	34.4±9.4	30.3 ±7.01	34.4±9.4	34.4±9.4	37.4 ±10.4
Gender (F[c]: M[d]; ration)	10 F: 10 M	4 F: 4 M	6 F: 6 M	6 F: 6 M	3 F: 4 M
Handedness (R[e]: L[f]: A[g])	18 R: 2 L	6 R: 2 L	12 R	7 R	7 R
Education Level (G[h]: U[I]; %)	82% G: 18% U	82% G: 18% U	100% G	100% G	100% G

[a] Mean,
[b] Standard Deviation
[c] Female
[d] Male
[e] Right
[f] Left
[g] Ambidextrous
[h] Graduate
[I] Undergraduate

minimize discomfort and posterior side-effects. Both protocols rely on the tDCS mechanism of action, in which depolarization of the synaptic membrane occurs because of the excitation under the anode. There is a decrease in the excitability under the cathode due to membrane hyperpolarization (see Fig. 4).

2.1.4. Data analysis

The data were pre-processed by using Neuroguide (Applied Neuroscience, Inc) and further analyzed by SPSS (IBM SPSS statistical analysis software). After the preprocessing steps, the data was first normalized (Z score) individually for each qEEG data session. Three standard deviations from the mean were marked in red and blue, which indicated abnormal activities of hyperactivation and hypoactivation, respectively. Second, non-parametric alternative to paired t-test - Wilcoxon Signed-Ranks Test – was conducted based on group means to inspect the effect of tDCS on qEEG spectral power (absolute power), brain connectivity indexes (amplitude asymmetry, coherence, and phase lag), and clinical inventories (BAI, BDI, PANAS, PSS) among the three time points (BEFORE tDCS, SHAM, and AFTER tDCS). These analyses complemented the information provided by the individual Z scores. In addition, a non-parametric alternative to independent sample t-test - Mann Whitney U - was conducted to detect the significant differences between the clinical and control groups and between eyes-closed and eyes-open protocols. Furthermore, a non-parametric alternative to Pearson correlation - Spearman's correlation - was employed to identify the relationship between the significant qEEG abnormalities (altered spectral power in left ACC) and the power bands among both groups. Finally, the error bars plots were calculated based on standard error which provide the variability/uncertainty measure of the data.

3. Results

3.1. The difference in absolute spectral power over F7 before and after the tDCS intervention

A non-parametric alternative to paired t-test was performed to examine the difference in absolute spectral power inspected by qEEG over time. The analysis results demonstrated a significant reduction over F7 across all frequency bands, W = -2.521, p= 0.012, such that "BEFORE TDCS" exhibited a higher mean (M = 48.34; SD = 61.2) than "AFTER TDCS" (M = 21.78; SD = 40.6), see Fig. 5.

3.1.1. The difference in absolute spectral power over alpha band before and after the tDCS intervention

A non-parametric alternative to the paired t-test was performed to examine the difference in alpha spectral power inspected by qEEG over time. The results showed a significant reduction over the alpha band in "AFTER TDCS" treatment (W = -3.288; p=0.00), as compared to that from the other two time points, particularly between "SHAM" (M = 59.48; SD = 35.8) and "AFTER TDCS" (M = 46.89; SD = 29.9) (see Fig. 6).

3.1.2. The difference in clinical inventories before and after the tDCS intervention

A non-parametric alternative to the paired t-test was also conducted to examine the effect of tDCS in the clinical self-reported measurements over time. The clinical inventories (BAI, BDI and PANAS-PA) exhibited significant differences: the Beck Anxiety Inventory (BAI) showed a significant difference between "BEFORE TDCS" and "AFTER TDCS", W= -2.524; p=0.012, in which "BEFORE TDCS" demonstrated a higher mean (M = 34.1; SD = 9.2) compared to the "AFTER TDCS" (M = 18.0; SD = 6.65); the Positive Affect Schedule (PANAS-PA) showed a significant difference between "BEFORE" and "AFTER TDCS" W = -2.371, p= 0.018,

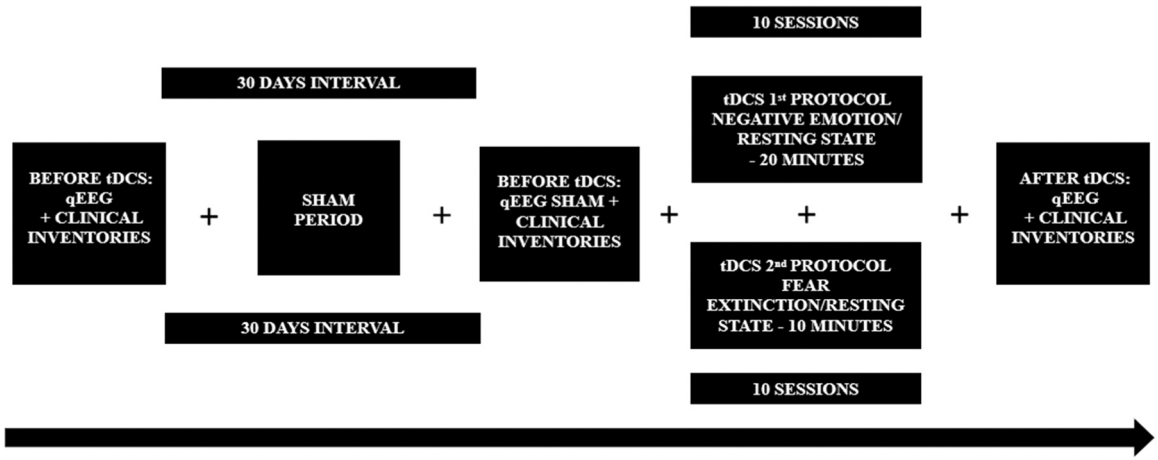

Fig. 2. Schematic paradigm of the experimental procedures.

Table 2
Stimulation parameters for tDCS protocols.

	anode/cathode (montage)	density (mA/cm^2)	duration (min)	outcome
Negative emotion modulation	F6/Fp1	1.5 mA	20 m	BDI/PANAS
Fear modulation	AF3/TP1	2 mA	10 m	BAI/PANAS

in which "BEFORE" had a lower mean (M = 23.62; SD = 6.63) compared to "AFTER TDCS" (M = 28.37; *SD* = 7.44); the Beck Depression Inventory (BDI) showed a significant decrease between SHAM (M= 32.3; SD = 9.00) and "AFTER tDCS" (M = 15.1; SD = 7.05; W= -2.035, p=0.042). Finally, the Negative Affect Scale (PANAS-NA) and the Perceived Stress Scale (PSS) only demonstrated a qualitative decrease in scores and no significant effect was found after tDCS (see Fig. 7 for significant results).

3.1.3. *Differences in coherence connectivity indexes before and after the tDCS intervention*

A non-parametric alternative to the paired t-test was conducted to examine the effect of tDCS in coherence connectivity index over time. A W-test was conducted to inspect the significant differences over four frequency bands (delta, theta, alpha, beta) between "BEFORE TDCS" and "AFTER TDCS", FP1-F7, W = −4.273; p=0.042, p≤0.05 (see Fig. 8).

3.1.4. *Differences in phase lag connectivity indexes before and after the tDCS intervention*

A non-parametric alternative to the paired t-test was conducted to examine the effect of tDCS in phase lag connectivity index over time. A W-test was conducted to inspect the significant differences over the four frequency bands (from the left to the right: delta, theta, alpha, beta) between "BEFORE" and "AFTER TDCS," W = -2.319; p=0.02, p≤0.05 (see Fig. 9).

3.1.5. *qEEG difference between the clinical and control group*

A non-parametric alternative to the independent sample t-test was conducted to investigate the difference between the clinical (n=8) and control groups (n=12). A significant difference was detected between the two groups in spectral power over the left anterior cingulate cortex, U = -1.964; p= 0.050, in which normal controls without treatment showed higher means (M = 19.144; SD = 26.4) as compared to the clinical group post-intervention (M = 1.48; SD = 0.50). The results are

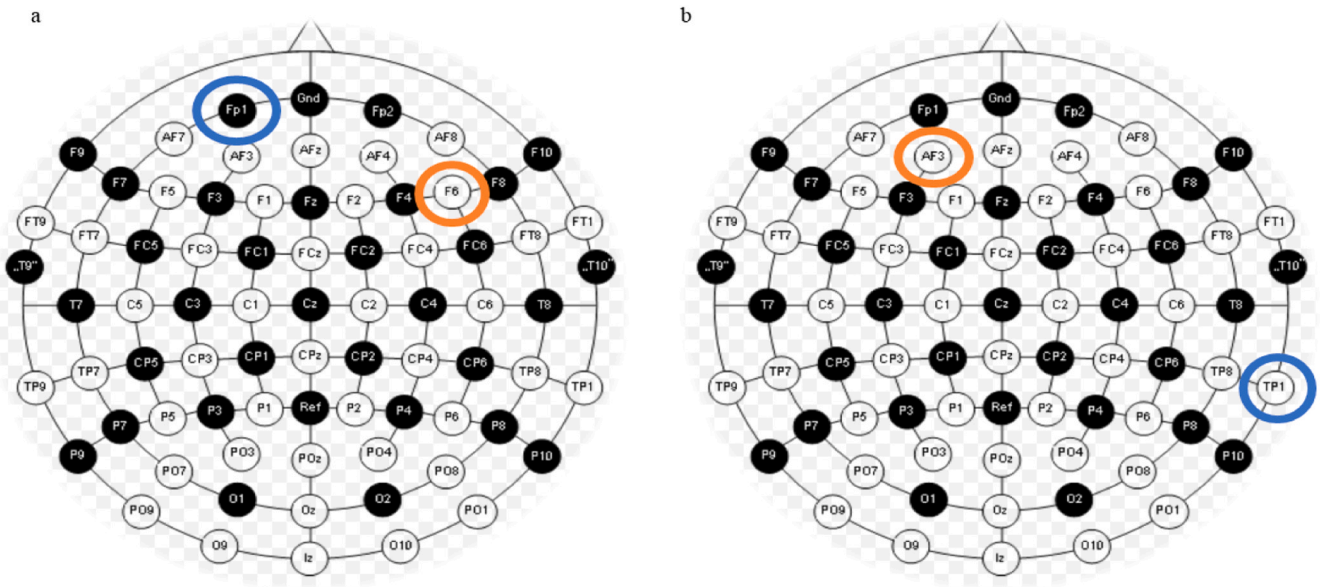

Fig. 3. Schematic montage for tDCS neuromodulation protocols. The figure presents the two montages involved in (a) negative emotion modulation (1.5 mA x 20 minutes), and (b) fear extinction (2 mA x 10 minutes) protocols. The orange circle represents the anode, while the blue circle represents the cathode.

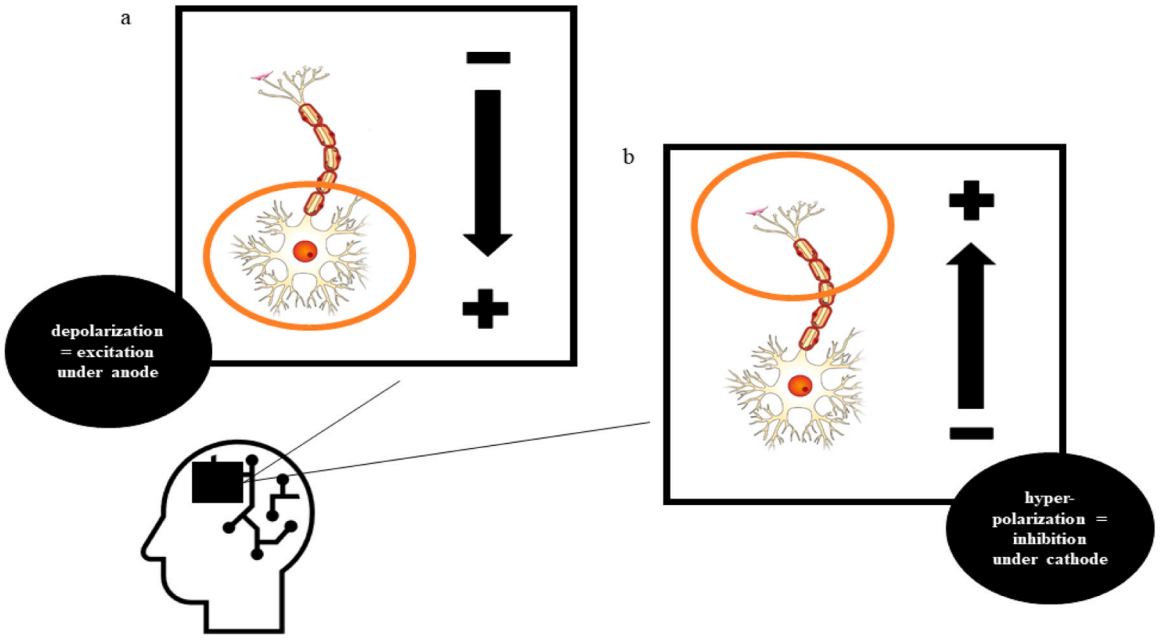

Fig. 4. tDCS mechanism of action. From depolarization (a) to hyperpolarization (b) in the synaptic membrane.

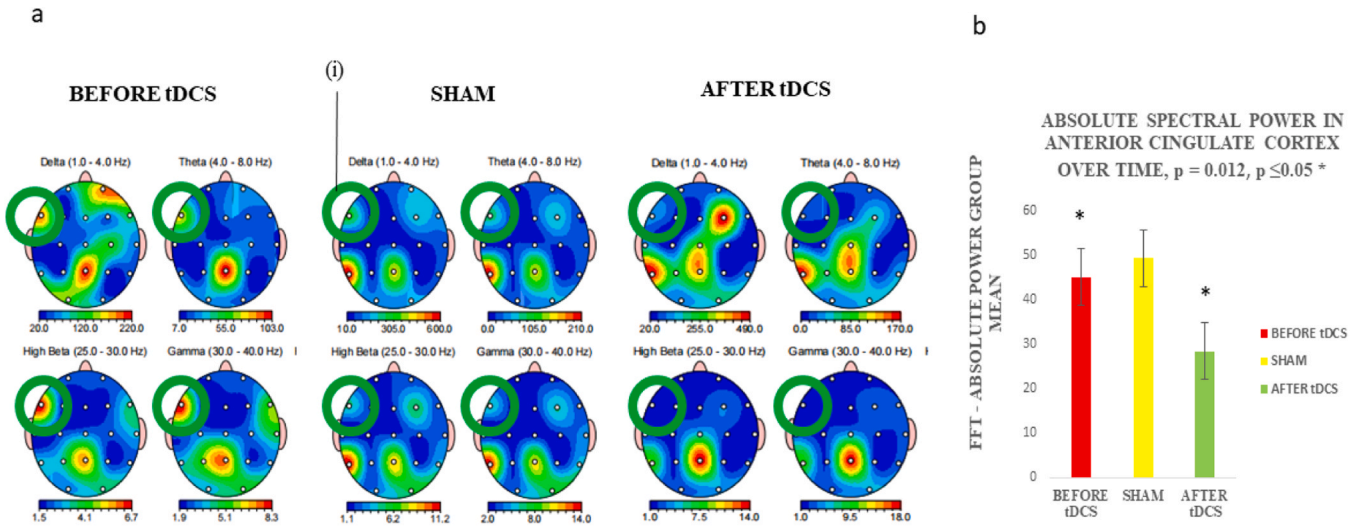

Fig. 5. The adjustment of absolute spectral power in the left ACC. Figure (a) represents the topographical mapping of qEEG spectral power, in which the green circle (i) highlights the target region over time. The bar graph in Figure (b) illustrates a significant difference between BEFORE tDCS (red) and AFTER tDCS (green) in the left ACC, p=0.012, p≤0.05. The error bars were calculated based on standard error of the distribution.

visualized in Fig. 10.

(p= 0.050), and respective topographic qEEG mappings illustrate the absolute spectral power changes located at the left ACC, marked by green circles (i) between the control group (b) and clinical group (c). The error bars were calculated based on standard error of the distribution.

3.1.6. Correlations between spectral power bands with clinical and control groups

A non-parametric alternative to the Pearson correlation - Spearman's correlation - was conducted to investigate the relationship between spectral power bands with the clinical and control groups (see Fig. 10). A significant correlation was found between the left ACC in the clinical group and theta band "AFTER TDCS" (r=0.554, ≤0.05), and between the left ACC in the clinical group with alpha band during the three stages of qEEG measurements: "BEFORE tDCS" (r= 0.998, p≤0.01), "SHAM" (r=0.932, p p≤0.01), "AFTER tDCS" (r=0.874; p≤0.01), see Table 3a. A significant correlation was also found between the control group without tDCS with the theta band (r=0.493, p≤0.05), and the alpha band (r=0.947, p≤0.01), see Table 3b.

4. Discussion and conclusion

Continuous challenges have been imposed on mental health science by Anxiety and Depression disorders as the most prevalent and debilitating psychiatric conditions worldwide.

The demand for new therapeutic solutions in affective disorders has given space to the refinement of tDCS protocols, being reported as the most safe and well-tolerated technique for the treatment of Affective Disorders. However, the lack of evidence with studies employing repeated sessions in anxiety disorders and the resistant comorbid symptoms which prevail after conventional interventions, motivated this study. Besides, the substantial gap of EEG evidence in the literature

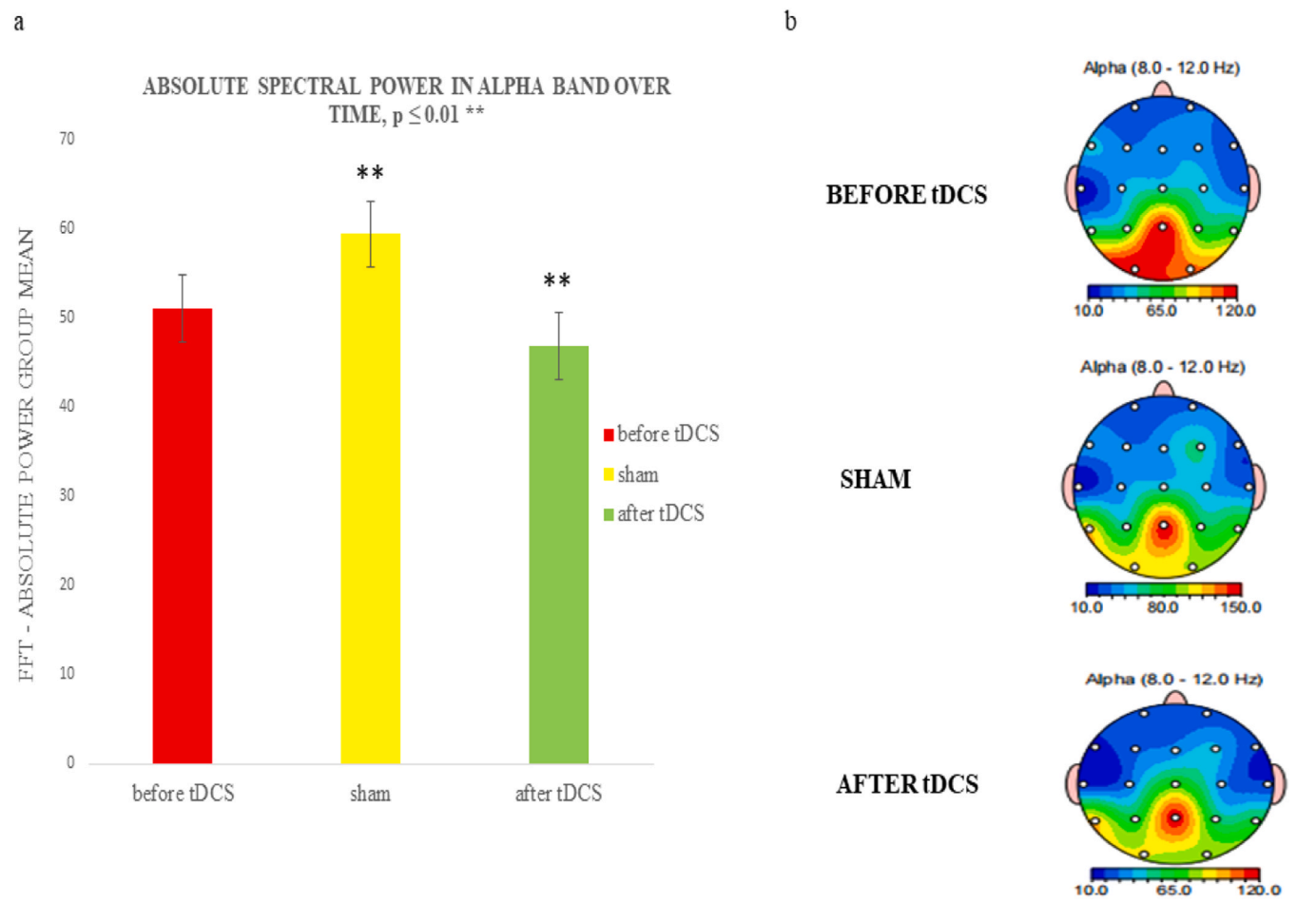

Fig. 6. The adjustment of the alpha frequency band over time. The bar graph (a) illustrates a significant difference between SHAM period (yellow) and AFTER tDCS (green) in the alpha band, p= 0.001, while the group means absolute spectral power (b) are represented by alpha band over time (BEFORE tDCS, SHAM, AFTER tDCS). The error bars were calculated based on standard error of the distribution.

confirming the impact of tDCS neuromodulation in both conditions added an innovative aspect to this research work.

In this study, qEEG and tDCS were combined to offer an in-depth investigation of neural signatures involved in dysfunctional regulation and target the brain regions responsible for affective regulative dynamics. In detail, qEEG offers a comprehensive Z-score strategy to characterize absolute power and connectivity abnormalities whereas tDCS provides a non-invasive and patient-friendly methodology for brain stimulation.

First, findings in relation to absolute power abnormalities elicited by left ACC and alpha altered spectral power over parietal regions and respective normalization over time showed that these regions might potentially be involved in the neuromodulation of affective responses, while playing a critical role in maintaining anxiety-depression comorbidity. For example, significantly decreased absolute spectral power along the left anterior cingulate cortex (ACC) was detected for the patient group after the tDCS intervention. In particular, ACC seems to be involved in the maintenance of depressive cycle, being responsible to particularly regulate the negative emotionality (Vergallito et al., 2018b), (B. AL, S. Saxena, M. MA, F. LA, H. ML, and B. LR, 2001)– (Etkin et al., 2015). Our findings are consistent with reports from the influential model proposed by Philipps et al (Phillips et al., 2003). indicating that ACC is linked to the dorsal and ventral systems of the prefrontal cortex responsible for the regulatory dynamics of emotional processing, affective regulation states, and consequent behaviour adjustment. Our results also illustrated a decreased abnormal alpha band over the posterior regions after tDCS neuromodulation. Interestingly, the alpha-band normalization and alpha symmetry training sustained by the literature seem to play an essential role in decreasing comorbid anxiety-depressive symptoms by reducing the existing imbalances over the frontal and parietal regions (Jaworska et al., 2012).

Of note, the brain connectivity results showed a significant decrease in coherence index (CI) over the left ACC after tDCS stimulation. The CI findings were significantly affected by tDCS intervention, thus demonstrating an improvement of coherence over the left anterior regions, specifically over F1-F7 and F3-F7. Interestingly, the normalization of coherence indexes was also detected over all bands, such as delta, theta, alpha, and beta. As previous research (Bradley et al., 2010) proposed, the Z-score adjustment represents a promising strategy to promote higher network flexibility during stressful events. Likewise, Bradley and colleagues confirmed the association between lower coherence over the left hemisphere and higher vulnerability to mood instability. Therefore, significant improvements were observed in the coherence index over the left anterior regions, which supports an improvement in attentional and executive resources to enhance flexibility, reduce overthinking patterns, and increase positive affection and motivation.

In addition, the phase lag index (PLI) demonstrated significant improvement after tDCS in the fronto-cingulate network, specifically in FP1-F7 connections. Other phase lag connections presented qualitative enhancements; however, they did not present significant differences. The phase lag index informs about the speed of the synchronization and the efficiency of the network. Previous research (Olbrich et al., 2014) proposed that abnormal phase-lag synchronization was identified in major depression (MDD) and located explicitly over the anterior

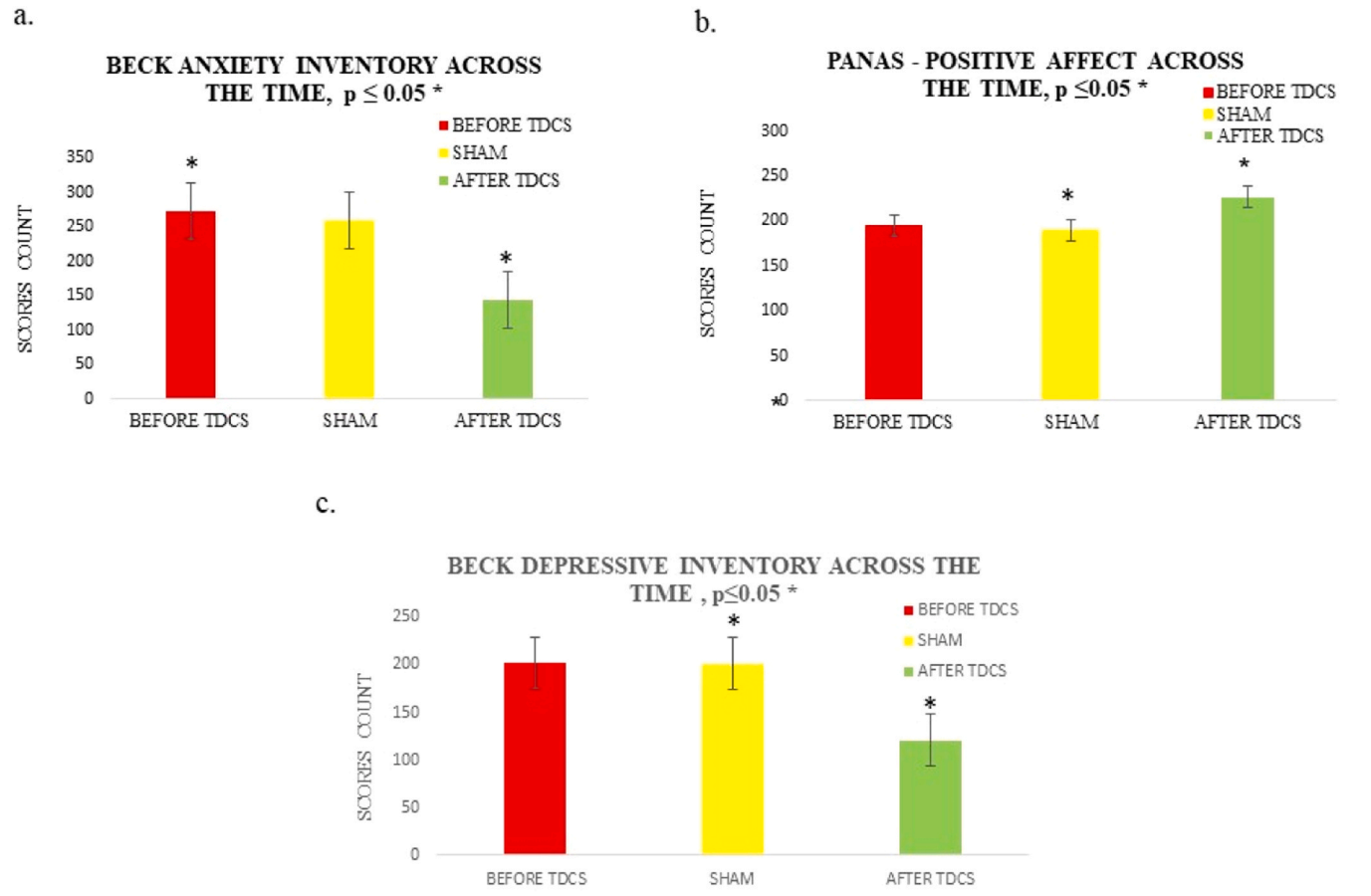

Fig. 7. Significant differences in clinical symptoms over time. Mean scores obtained in (a) Beck Anxiety Inventory between BEFORE tDCS (red) and AFTER tDCS (green), p= 0.012, p≤0.05; in (b) Positive Affect Scale – Positive Affect (p= 0.018; p≤0.05) between BEFORE tDCS (red) and AFTER tDCS (green); and (c) Beck Depression Inventory between SHAM (yellow) and AFTER tDCS (green), p=0.042, p≤0.05).The error bars were calculated based on standard error of the distribution.

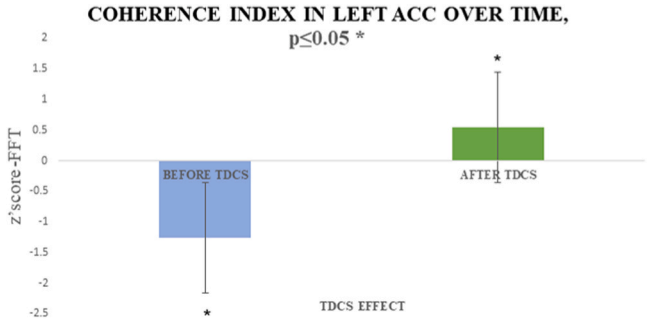

Fig. 8. Coherence connectivity index over time. The bar graph illustrates significant differences in coherence connectivity index in frontal-cingulate networks among the periods BEFORE and AFTER tDCS (p=0.042, p≤0.05). The error bars were calculated based on standard error of the distribution.

cingulate cortex and dorsolateral prefrontal cortex. Besides the depression symptoms, anxiety symptoms have also been linked to an increase in phase synchronization over resting-state theta bands in patients with anxiety compared to the controls (Xing et al., 2017). The previous evidence suggests that resting-state theta bands generated by ACC are commonly involved in internalization conditions. Therefore, the improvement in phase synchronization located over the ACC and DLPFC is supported by the existing literature (Olbrich et al., 2014), (Xing et al., 2017) and explains the perceived improvement of attention and executive functions reported by the clinical group.

In line with the power and functional connectivity results, significant improvements were also detected in the BAI, BDI PANAS-PA after the tDCS intervention for the patient groups. Extensive research in tDCS has categorized inventories including BDI, BAI (Bardhoshi et al., 2016), (Beck and Steer, 1988), and PANAS as robust instruments to measure anxiety and depression symptoms (Watson, 1988), while PSS has been chosen as the most widely used instrument to measure perceived stress (S. Publications, 1983). Considering the current findings, the decrease in anxiety levels (BAI) after tDCS neuromodulation seems to be supported by the parietal alpha signature identified for the patient group. Interestingly, a previous study (Jaworska et al., 2012) testing alpha power and alpha asymmetry also confirmed a link between parietal alpha index with emotional arousal and anxiety in depressed females. Concurrently, improvement of positive affection (PANAS-PA) in depressed patients after tDCS neuromodulation has been associated with modulation of the left ACC because it is a region linked to approach strategies, motivation, and positive affection (Davidson et al., 1990). Besides the consistent improvements, our study did not find any significant tDCS effect in perceived stress and negative affect scores, only detecting a qualitative decrease over time. These findings might suggest the need for an increased number of repetitive tDCS sessions to target a broad set of negative emotions and task-activation protocols to selectively modulate the negative emotionality, instead of using the selected protocols at rest. Of note, the range of negative emotions integrated in the negative affect scale expands the focus of fear or sadness, integrating a more complex layer of negative emotionality.

Reduced abnormal activity over the left ACC was detected for the patient group after treatment compared to the control group, as expected since the control group was not exposed to tDCS

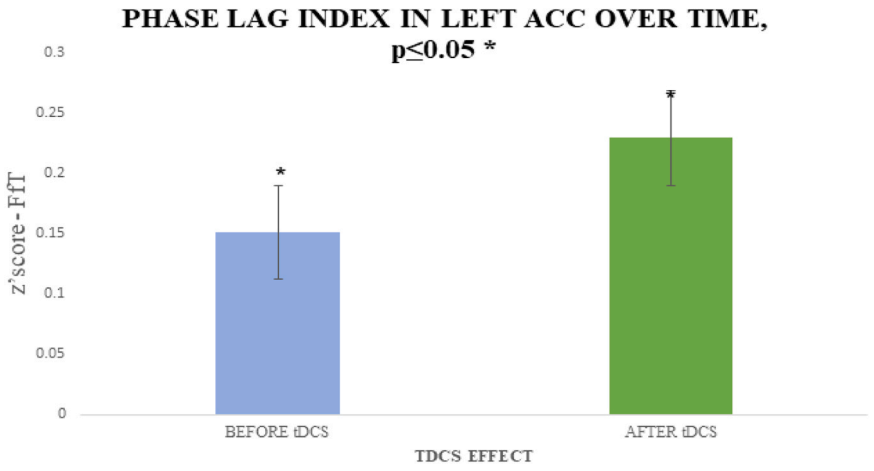

Fig. 9. Phase Lag Connectivity Index over time. The bar graph illustrates significant differences in phase lag index in frontal-cingulate networks between BEFORE tDCS and AFTER tDCS (p=0.02 ≤0.05). The error bars were calculated based on standard error of the distribution.

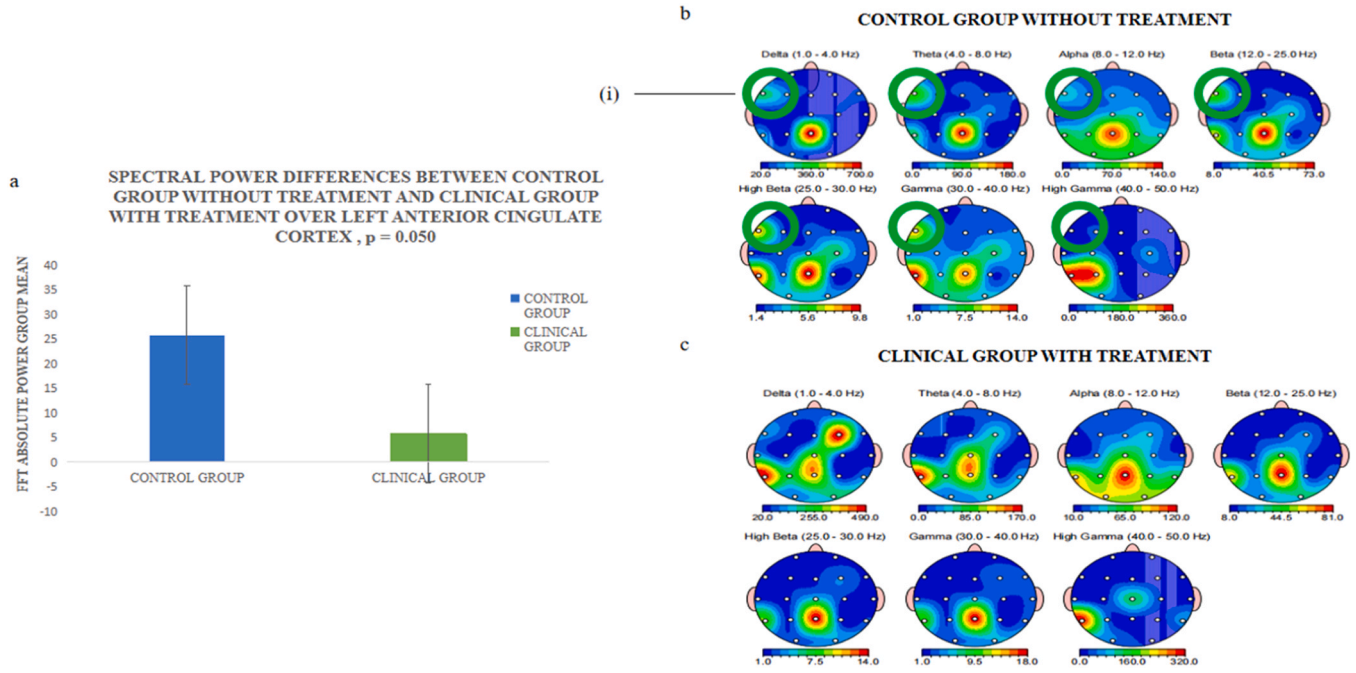

Fig. 10. Absolute spectral power differences between the clinical and control groups. The bar graph (a) represents spectral power differences between the control group and the clinical group in the left ACC (p= 0.050).

Table 3a
Correlations between spectral power bands and left ACC in clinical group after tDCS.

left ACC in clinical group	theta power, after tDCS	alpha power, before tDCS; SHAM; after tDCS
Spearman's correlation	0.554*	0.998** 0.932** 0.874**
Sig. (2-tailed)	0.014	0.001

**r = Strong positive correlation
*r = Moderate positive correlation

Table 3b
Correlations between spectral power bands and left ACC in control group without tDCS.

left ACC in control group	theta power, qEEG without tDCS	alpha power, qEEG without tDCS
Spearman's correlation	0.493*	0.947**
Sig. (2-tailed)	0.032	0.001

**r = Strong positive correlation
*r = Moderate positive correlation

neuromodulation. Although the result informed that left ACC might play a pivotal role in distinguishing the patient group from the control group after treatment, the significant difference found between both groups was not very expressive, since two participants presented higher scores for anxiety and depression. These two participants did not qualify for the clinical group since the increased scores were reactive to the stressful work environment, and these symptoms were considered a functional reaction to the harmful environment and not due to a consistent dysfunctional emotional regulation pattern visible in other settings of daily life. These findings highlighting the key role of left ACC in regulatory dynamics are consistent with previous reports from Wacker et al. (2003) and Harmon-Jones (Harmon-Jones, 2004). Moreover, the

abnormal theta bands can also explain the ineffective pattern of affective response over the left ACC. Jaworska and colleagues (Jaworska et al., 2012) inspected the role of the ACC in depressed patients with comorbid anxiety and their findings suggest that the role of the rostral ACC-theta elicited during emotional processing and cognitive control could modulate affective responses. Therefore, the rostral ACC seems to perform a compensatory mechanism over fronto-cingulate networks to regulate emotional reactions in depressed patients.

In line with previous findings, the positive and moderate correlation observed between the clinical group and theta power band after the tDCS treatment seems to support the relationship discussed above while highlighting the theta power oscillations as a robust signature to target in the neuromodulation of affective responses, particularly those involved in comorbidity (Jaworska et al., 2012), (Xing et al., 2017). In more detail, the literature reported the role of the theta band involved in theta-beta oscillations responsible for recruiting executive and attentional control circuits to regulate emotional responses (Jaworska et al., 2012). In addition, the alpha power band was also positively correlated with the clinical group over the three different stages of qEEG examination, supporting the relationship between alpha band abnormalities and the prevalence of anxiety-depressive symptoms maintained by the frontal-parietal imbalances (Jaworska et al., 2012), and the potential need to keep monitoring the alpha band as a follow-up in the next stage of the clinical trial. As expected, the control group without treatment was correlated with theta and alpha band abnormalities. Therefore, the theta band elicited after treatment in the clinical group and not in the previous stages might suggest the key role of tDCS in helping to normalize the power imbalances responsible to maintain the internalizing states, however, this effect might be transient and short duration and more sessions combined with specific protocols might be considered to address the rumination and overthinking patterns quite prevalent in internalizing states. A larger sample size might also be required to inspect this relationship in more detail.

Finally, a comparison between resting state protocols (eyes-open and eyes-closed) was conducted to understand the reliability of inspecting the target mechanisms. The findings did not inform any particular sensitivity between protocols; however, the results might offer some insights regarding the implementation of these resting state protocols in tDCS settings. While there is no significant difference found between protocols, the qualitative results suggested that eyes-closed protocol might offer higher sensitivity for conducting an inspection of neural mechanisms over anterior-posterior mechanisms. According to the review, the eyes-closed protocol is less affected by the modulation of attention resources, while the eyes-open approach might present a relevant complementary approach to investigate neural mechanisms modulated by attention and executive resources. Besides, the third model proposed by Bickson & Rahman (Bikson and Rahman, 2013) – input selectivity – in which the authors propose that the network is enhanced at the cost of another might encounter more conditions to perform in eyes-closed protocols, since the eyes-closed protocols might be less impacted by attentional resources and less susceptible to be driven by the hypervigilance mechanism to thread cues.

Collectively, our findings showed the influence of tDCS in modulating the impaired regulatory dynamics encoded in the left anterior cingulate cortex and reducing abnormal spectral power activity over the alpha band over central posterior regions. Concurrently, the tDCS enhances frontal-parietal intra-hemispheric connections to decrease the imbalances within the left hemisphere while making information processing more flexible and efficient over anterior frontal connections. According to the influential theorization of regulatory dynamics (Phillips et al., 2003), tDCS influences the ventral neural systems responsible for emotional processing, while regulating fear and negative affect responses. Moreover, tDCS also modulates the dorsal neural systems specialized in executive functions to facilitate emotional regulation and coordinate the respective behaviour adjustment. Thus, this study manifests an effort to address highly prevalent psychiatric symptoms, while investigating the reliability of combined qEEG-tDCS to reduce altered neural signatures, such as the abnormal power activity and connectivity efficiency in resting-state networks. Moreover, the normalization of the altered neural signatures seems to translate into a consistent reduction of comorbid symptoms which potentially expands the understanding of the plasticity in regulatory circuits responsible for the maintenance of affective disorders.

To sum up, this study offers a tDCS sham approach combined with quantitative electroencephalogram standardization as a feasible strategy to deliver brain stimulation while addressing comorbidities that co-occur in affective disorders. However, some limitations should be emphasized and carefully addressed in future research work. First, the relationship between qEEG and clinical effects is only correlational, and causality analysis might be employed in the next research directions to inspect the causal relationship between variables. The sample size should be enlarged to achieve more robust evidence about qEEG abnormalities in affective disorders. In addition, an activation task embedded in a classic or in an immersive virtual reality paradigm might be considered during the qEEG measurements to target other layers of negative emotions before and after the neuromodulation. Furthermore, novel tDCS techniques should be developed to target the entire brain network rather than those restricted to two network sites (anodal and cathodal). Finally, the current study relies on a sham-controlled design integrated into a quasi-experimental design. Randomized controlled trials (RCT) should be addressed by future investigations and larger clinical trials.

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CRediT authorship contribution statement

Fei Gao: Writing – review & editing. **Tania Alexandra Couto:** Conceptualization, Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Davis C. Lak:** Data curation. **Zhen Yuan:** Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability

The qEEG-tDCS data used to support the findings of this study are available from the corresponding author upon request, requiring a formal data sharing agreement due to the privacy issues of clinical data.

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