Study Protocol

Title	The effects of High-definition transcranial direct current stimulation on cognitive bias among youth with social anxiety symptoms
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Literature review

Social anxiety is the third most common mental health issue after depression and alcohol abuse (Aune et al., 2022), particularly for youth aged from 15 to 24 (Tang et al., 2022). From the perspective of the information processing, cognitive bias is the main cause of the occurrence and maintenance of social anxiety symptoms in youth, encompassing three aspects: attention bias, interpretation bias, and memory bias (Amir & Bomyea, 2010; Kuckertz & Amir, 2014). Specifically, youth with attention bias tend to focus on the threat-related cues in the social environment (attentional vigilance), and then quickly shift their attention away (attentional avoidance). Building on attention bias, interpretation bias may lead socially anxious youth to interpret ambiguous social information negatively and attributions negative events to internal causes (Amir & Bomyea, 2010; Haller et al., 2016; Martinelli et al., 2022). In addition, memory bias refers to the tendency to recall or recognize negative stimuli, especially self-related negative information (Kuckertz & Amir, 2014; Morgan, 2010). These three cognitive biases form a self-reinforcing circle, perpetuating and exacerbating social anxiety symptoms (Nikolić, 2020).

Current treatments for cognitive bias, such as cognitive bias modification, can only yield a significant but small and unstable effect. (Enock et al., 2014; Heeren et al., 2015; Liu et al., 2017). As a result, recent research has shifted toward targeting the underlying mechanisms to achieve more robust reductions in cognitive bias, particularly at the neural circuit level (Heeren et al., 2016). High-definition transcranial direct current stimulation (HD-tDCS) is a non-invasive brain stimulation technique that delivers direct current through electrodes placed on the scalp. This current induces polarization of neuron cell membranes, thereby enhancing or inhibiting cortical activity (Kuo et al., 2013; Stein et al., 2020). For individuals with social anxiety, they often exhibit reduced regulatory control of prefrontal cortex (PFC) over the limbic system, particularly in the left dorsolateral prefrontal cortex (DLPFC) (Cremers & Roelofs, 2016). Therefore, previous tDCS studies adopted anodal tDCS to the left DLPFC and demonstrated that both attention, interpretation and memory bias in clinical populations can be significantly improved (Heeren et al., 2016; Nejati et al., 2022; Nejati et al., 2021).

However, most intervention studies just applied online tDCS stimulation to modify cognitive bias while overlooking its offline effects. Compared to short-term online effects, long-term offline effects are crucial for reducing intervention dependence and restoring normal social functioning. Moreover, previous studies have lacked direct tDCS interventions targeting socially anxious youth, resulting in insufficient consideration of the core characteristics and specific cognitive processing patterns associated with social anxiety. Specifically, regarding attention bias, prior studies has primarily focused on attentional vigilance while neglecting attentional avoidance (Jafari et al., 2021; Nejati & Estaji, 2024), a key characteristic of youth with social anxiety (Morales et al., 2015). In terms of interpretation bias, previous studies have failed to incorporate social contexts that may trigger negative evaluations into their measurement paradigms (Nejati et al., 2021), making it difficult to generalize findings from other clinical populations to socially anxious youth. Regarding memory bias, individuals with social anxiety tend to focus on their own performance in social situations, making them more likely to encode and retrieve self-referential negative memories. Thus, it is essential to examine tDCS effects on negative memory bias within a self-referential encoding framework. To address these gaps, the present study implemented offline tDCS stimulation and adjusted the stimulation protocol to enhance sustained intervention effects, and adopted a group-specific assessment paradigm to improve the precision of cognitive bias evaluation in socially anxious youth. In addition, while many studies have examined the effects of tDCS on attention, interpretation or memory bias individually (Jafari et al., 2021; Nejati & Estaji, 2024; Sanchez-Lopez et al., 2018; Winker et al., 2019), its influence on psychopathology symptoms remains underexplored. Given this, the current study utilized HD-tDCS as an intervention to explore changes in social anxiety symptoms.

In current study, we adopted a comparative intervention study with randomized controlled in which we delivered multiple courses of offline anodal HD-tDCS over the left DLPFC. The aim of this study was to explore the offline impact of HD-tDCS on cognitive bias (including attention, interpretation and memory bias) among youth with social anxiety symptoms. In addition, the researchers sought to further investigate the impact of tDCS stimulation on social anxiety. We hypothesized that anodal tDCS would activates the left DLPFC to facilitate cognitive control of threat-related information among youth with social anxiety, thereby reducing threat attention, interpretation and memory bias, and even improving social anxiety symptoms.

Method

Participants

This study recruited youth with social anxiety symptoms from the South China Normal University in Guangdong Province, China. Participants who were: 1) aged 15 to 24 years; 2) scored above 55 and below 95 on the Liebowitz Social Anxiety Scale (the cut-off score for social anxiety in the Chinese version (Liebowitz, 1987); 3) scored below 5 on the Patient Health Questionnaire (Kroenke et al., 2001) were eligible. Those with 1) any psychiatric disorders; 2) current or past diseases or injuries related to the brain; 3) medical pumps, pacemakers and cochlear implants in the body; 4) current pharmacological or mental treatments; 5) tDCS or TMS experiences over past year were also excluded.

Randomized and blinding

Participants were randomly assigned to either tDCS or sham groups using a computer-generated randomization table, with a 1:1 allocation ratio. Participants remained blinded to treatment conditions throughout the intervention period. Researchers only received allocation information immediately prior to procedure implementation.

Interventions and study procedures

The whole HD-tDCS intervention process was 10 sessions for 5 consecutive days (20 minutes each session and 2 sessions a day). All participants received active or sham HD-tDCS stimulation via a device from Soterix Medical, Inc (Woodbridge, NJ, USA). According to the 10/20 International EEG System, a “4 x 1 ring set-up” was placed in F3, FP1, FT7, C3 and FZ. F3 is the central anode electrode, which refers to the left

DLPFC, surrounded by the other 4 return cathode electrodes (Heeren et al., 2016; Jafari et al., 2021; Liu et al., 2023; Müller et al., 2022; Nejati et al., 2021; Parlikar et al., 2021). Both the tDCS group and the sham group were given electric current at the same electrode position. Normally, the current gradually rose to 2mA for the first 30 seconds, then continued for 20 minutes and then dropping to 0mA at the end of the stimulation. In the sham group, the current ramped up to 2mA for 30 seconds at the start and end of the stimulus and then ramped down without the participants' being aware of it, generating the same sensation as the active condition. During the intervention, participants were asked to maintain a relaxed and immobile posture.

Prior to the study, participants completed an enrollment screening questionnaire to determine their suitability for tDCS intervention. Participants who met the inclusion criteria were randomly divided into the tDCS and the sham group. Both two groups received 10 sessions of stimulation with 3 hours intervals between daily sessions (2 sessions daily, 5 days in total). Psychological symptoms (including social anxiety symptoms, cognitive bias and trait anxiety) and cognitive bias task performance (including dot probe task, word-sentence association paradigm, free recall and recognition task) were assessed before the first tDCS stimulation (pre-test) and after the last tDCS stimulation (post-test). According to the Latin square, the order of the three cognitive bias tasks was randomly counterbalanced among the participants. Finally, in order to ensure the safety of the tDCS stimulation and to compare the perception of this stimulation between different groups, a side-effect checklist was filled out by the participants after each session of stimulation. In addition, psychological symptoms were assessed again at 4 weeks follow-up. Participants in the entire experiment were rewarded with 280 RMB.

Sample Size Calculation

The required sample size was calculated using G*Power software. Based on repeated measures analysis of variance, the sample size for each group was at least 27 (effect size = 0.25, = 0.05, power = 0.95). To avoid the impact caused by loss, an additional 10 people were included in each group, that is, 37 people. A total of 74 people were recruited in two groups.

Assessments and outcomes

Questionnaires

The Liebowitz Social Anxiety Scale (LSAS) was used to distinguish participants with social anxiety (defined as an LSAS score ⩾ 55) and assess the changes of anxiety symptoms between pre- and post-tDCS intervention. This scale involves 24 Likert-items with higher score indicating severe social anxiety (Gong et al., 2023; Liebowitz, 1987). The Patient Health Questionnaire (PHQ-9) was only used to screen those without depression. The total score of the nine items was calculated, ranging from 0 to 27, with a cut-off score of 5 (Kroenke et al., 2001; Wang et al., 2014). In addition, participants also completed the Negative cognitive processing bias questionnaire (NCPBQ) and the Spielberger Trait Anxiety Inventory - Trait (STAI-T). The NCPBQ consists of 17 items divided into three subscales with three lie detection items: a 5-item attention bias, a 5-item interpretation bias and a 4-item memory bias. It was measured using a 5-point Likert scale from 1 (“disagree completely”) to 5 (“agree completely”). A higher score represented a higher level of cognitive bias (Miao et al., 2022; Zhang, 2015). The STAI-T is an inventory concerning and evaluating trait anxiety. There are 20 items with 4-point Likert style from 1 (“not at all”) to 4 (“very much”) (Spielberger et al., 1971). All of these questionnaires in this study have high reliability (Cronbach’s alpha > 0.80).

Some sociodemographic information was also collected, with sex, ethnicity, residence and only-child status as binary variables, and socio-economic level, relationship with parents and family harmony as continuous variables.

Dot probe task

Dot probe task is a common experimental paradigm for measuring attention bias (MacLeod et al., 1986). After appearance of a fixed cross in the center of the computer screen, a pair of faces appear side by side. Then, a probe appears on the location previously occupied by one of the two faces. Participants were asked to response the location of the probe as quickly as possible and press the appropriate keys to answer. If the probe was presented at a location that matched the attention, participants would response more quickly than the mismatched location. In current study, the experimental task included 40 facial stimuli (20 male and 20 female), forming 30 face pairs (20 neutral-angry and 10 neutral-neutral combinations). Based on valence scores from preliminary testing, angry faces were categorized as either high-intensity and low-intensity. All these faces were selected from the Chinese facial affective picture system (Gong et al., 2011). The number of trials for each type of stimuli location (left or right) and probe location (left or right) were equal. And a total of 120 trials were delivered in one block (120 trials = 30 face-pairs * 2 face locations * 2 probe locations). The fixed cross appeared for 500ms and then a pair of facial stimuli (neutral-angry or neutral-neutral) appeared for 500ms. The probe ("*") then appeared and remained until a response ("F" for left and "J" for right) was made. The inter-trial interval was 1500ms.

This study used the trail-level bias score (TL-BS) as a measure of the main outcomes of this task (Zvielli et al., 2015). TL-BS is an indicator of reaction time (RT), calculated by subtracting contiguous pairs of congruent trials (probe and emotional face appeared on the same location of the screen) from incongruent trials (probe and emotional face appeared on the opposite location of the screen). Positive scores represent a tendency to be alert and attentive to emotional faces, while negative scores represent avoidance of these stimuli. A score close to zero indicated a balance of attention to emotional and neutral faces. Specifically, there are 4 TL-BS parameters: (1) Mean $\text{TL-BS}_{\text{positive}}$ indicates the average value of TL-BSs > 0ms; (2) Mean $\text{TL-BS}_{\text{negative}}$ indicates the average value of TL-BSs < 0ms; (3) Peak $\text{TL-BS}_{\text{positive}}$ indicates the maximum TL-BS value; (4) Peak $\text{TL-BS}_{\text{negative}}$ indicates the minimum TL-BS value.

Word sentence association paradigm

Word sentence association paradigm (WSAP) was used to assess the interpretation bias in youth with social anxiety (Beard & Amir, 2009). Following the appearance of a fixed cross (500ms), an ambiguous sentence describing the social situation would be presented in the center of the screen (2000ms). Then, a word would appear at random for 500ms: the word might imply a threatening interpretation (e.g., "boring" or "rejection"), or imply a benign one (e.g., "captivating" or "accepted"). Participants were asked to decide whether the word was related to the ambiguous sentence ("F" for relevant and “J” for irrelevant). There were 30 ambiguous sentences in a block, corresponding to 30 threatening words and 30 benign words, for a total of 60 word-sentence pairs. The word-sentence pairs would be presented in random order, and no pairs were repeated. This study calculated the percentage of threaten and benign interpretations that participants endorsed as being related to the sentence, which was prescribed as the primary result of interpretation bias. We also recorded the response time and calculated the bias index (BI): threat BI = response times (reject threat – endorse threat) and benign BI = response times (endorse benign – reject benign).

Free recall and recognition task

This study used free recall and recognition task to assess memory bias by the number of positive/negative words remembered. First, in the “encoding phase”, after a short time for a fixed cross (500ms), 30 words (15 positive words and 15 negative words) were presented randomly in turn (each word occurred for 2000ms). Participants were asked to read these words aloud and answer coding questions. The questions were divided into three categories, each corresponding to different coding conditions, including other-reference encoding (“To what extent does this word describe Jiong He?”. Jiong He is a very popular Chinese host), encoding with reference to the perception of self by others (“To what extent does this word describe what people think of you?”) and the self-reference encoding (“To what extent does this word describe you?”). Participants were asked to answer these questions using a four-point Likert scale, ranging from 1 (“Not at all”) to 4 (“Completely”). Each encoding conditions involved 10 words (5 positive words and 5 negative words), which were counter-balanced across participants. After this phase, there was a 3-minute distraction task, during which participants were guided through 40 calculation problems. They were then given 2 minutes to freely recall the words that had appeared before, regardless of the order of the words. Next, they completed a recognition task that contained 30 previously shown words and another 30 disturbing words. All of these words were presented for 4000ms in random sequence and selected from a glossary related to social anxiety, covering five aspects: communication skills, interpersonal perception, social performance, academic achievement, and social competence (Kalenzaga & Jouhaud, 2018). We assessed memory bias using accuracy rates for both positive and negative words in free recall and recognition tasks. Accuracy was calculated as: (number of correctly recalled/recognized words) ÷ (total number of words presented).

Data preparation and statistical analysis

Before formal analysis, this study addressed errors and outliers in the three tasks, respectively. Trials with incorrect reactions and with RTs greater than 3000ms, lower than 200ms, or greater than ±3 standard deviation were excluded when calculating each participants' task performances. We also treated attention, interpretation and memory bias index of ±3 standard deviation as extreme values.

Firstly, group differences across these two groups were measured using t-tests for scale and behavioral variables, and Chi-square tests for sociodemographic variables. To explore the intervention effect of tDCS on task performance, repeated-measures ANOVA were conducted with "group" (tDCS vs. sham group) as the between-subject and "time" (pre-test vs. post-test) as the within-subject factors. The outcomes of dot probe task, word sentence association paradigm and free recall and recognition task served as dependent variables. Before the ANOVA analysis, we used the Levene test to evaluate the homogeneity of variance of the data and the results showed that the data basically satisfied the homogeneity of variance hypothesis. The Bartlett and Mauchly's test were also performed to assess the sphericity of the data, and the degrees of freedom were modified by the Greenhouse-Geisser approach if needed. For subjective scale data, we employed 2-level linear mixed-effects regression, which included three fixed effects (group, time and the interaction between group and time) and one random effect (participants). All of these analyses were conducted using SPSS 25.0 and R software. The significance level was set at $p < 0.05$, and the marginal significance level was set at $p < 0.1$. Effect sizes were reported by the partial eta-squared (η^2) for ANOVA, and the Cohen's d for pairwise comparisons.

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