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Effects of combined theta burst stimulation and transcranial direct current stimulation of the dorsolateral prefrontal cortex on stress

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Highlights

- This study examined the effects of combined left prefrontal intermittent theta-burst stimulation (iTBS) and bifrontal transcranial direct current stimulation (tDCS) on stress.
- Combining iTBS with tDCS did not attenuate the psychophysiological stress response.
- Concurrent tDCS and iTBS of the prefrontal cortex resulted in more pain and feelings of discomfort.

Abstract

Objective: Research suggests that the combination of different non-invasive brain stimulation techniques, such as intermittent theta-burst stimulation (iTBS) and transcranial direct current stimulation (tDCS), could enhance the effects of stimulation. Studies investigating the combination of tDCS and iTBS over the dorsolateral prefrontal cortex (DLPFC) are lacking. In this within-subjects study, we evaluated the additive effects of iTBS with tDCS on psychophysiological measures of stress.

Method: Sixty-eight healthy individuals were submitted to a bifrontal tDCS+iTBS and sham tDCS+iTBS protocol targeting the DLPFC with a one-week interval. The Maastricht Acute Stress Test was used to activate the stress system after stimulation. Stress reactivity and recovery were assessed using physiological and self-report measures.

Results: The stressor evoked significant psychophysiological changes in both stimulation conditions. However, no evidence was found for differences between them in stress reactivity and recovery. Participants reported more pain and feelings of discomfort to the bifrontal tDCS+iTBS protocol.

Conclusion: In this study set-up, iTBS plus tDCS was not superior to iTBS in downregulating stress in healthy subjects.

Significance: There is no evidence for an effect of combined tDCS-iTBS of the DLPFC on stress according to the parameters employed in our study. Future studies should explore other stimulation parameters, additive approaches and/or neurobiological markers.

Keywords: Non-invasive Brain Stimulation, Transcranial Direct Current Stimulation (tDCS), intermittent Theta Burst Stimulation (iTBS), Dorsolateral Prefrontal Cortex (DLPFC), Stress, Psychophysiology.

1. Introduction

Non-invasive brain stimulation (NIBS) techniques targeting the dorsolateral prefrontal cortex (DLPFC) have been increasingly used in affective neurosciences and as non-pharmacological interventions in psychiatry (e.g., Brunoni et al., 2019; Brunoni and Vanderhasselt, 2014). One form of NIBS that has been extensively investigated is repetitive transcranial magnetic stimulation (rTMS; Baeken et al., 2019; Lefaucheur et al., 2014, 2020). A relatively new protocol of rTMS is theta burst stimulation (TBS), in which repeated bursts of high frequency (i.e., 50 Hz) stimulation are applied (Huang et al., 2005). Several studies have investigated the effects of intermittent TBS (iTBS) on motor cortex excitability (e.g., Huang et al., 2005), and, more recently, on the DLPFC for therapeutic purposes (e.g., Desmyter et al., 2016; Fitzgerald et al., 2020; Williams et al., 2018). iTBS has similar clinical efficacy for depression than standard rTMS protocols, within a remarkable short treatment duration (Blumberger et al., 2018; Mutz et al., 2019). However, despite the promising clinical outcomes, the effects of rTMS and iTBS remain relatively modest with treatment responses ranging from 35% to 50.6% (Baeken, 2018; Brunoni et al., 2017). Therefore, research focusing on ways to further improve clinical efficacy with rTMS are required.

In this context, several studies have examined the use of iTBS in combination with a subthreshold NIBS technique known as transcranial direct current stimulation (tDCS; e.g., Hasan et al., 2012; Tremblay et al., 2017). tDCS is an easy to apply and safe neuromodulation method with limited side effects (Bikson et al., 2016) and moderate clinical efficacy in the treatment of depression according to a recent meta-analysis (with response and remission rates of respectively 33.3% and 19.12%, see Razza et al., 2020). Whereas iTBS generates action potentials, tDCS modulates membrane potentials by shifting the neuronal firing threshold (i.e., it changes the neuronal polarity). Hence, although tDCS does not initiate action potentials, it is able to modulate the cortical excitability of the underlying brain regions within at least three minutes after stimulation onset (Nitsche and Paulus, 2000). Moreover, tDCS could potentiate the neural system by enhancing the effects of other interventions by inducing

synaptic plasticity via coactivation of the targeted network (i.e., functional targeting; Bikson and Rahman, 2013; Bikson et al., 2018; Jackson et al., 2016). According to the theory of functional targeting (e.g., Jackson et al., 2016), tDCS can boost plasticity when its combined with ongoing synaptic activity (i.e., during synaptic coactivation), for example when tDCS is combined with cognitive interventions that activate the same neural circuits (Sathappan et al., 2019) or transcranial magnetic stimulation (TMS; Jackson et al. 2016). Hence, tDCS could be more effective when combined with other forms of therapy.

Indeed, research on the effects of the combined use of tDCS and rTMS indicates that the combination of these techniques can result in larger effects compared to each technique by itself. In a pioneering study, Hasan and colleagues (2012) demonstrated that the effects of TBS on corticospinal excitability are modulated by the concurrent application of tDCS (Hasan et al., 2012). Specifically, whereas cathodal tDCS increased the facilitating effects of iTBS and decreased the inhibitory effect of continuous TBS (cTBS) on cortical excitability, anodal tDCS reversed the effects of cTBS but did not significantly affect iTBS (Hasan et al., 2012). Conversely, Park and colleagues (2014) reported decreased motor cortex excitability and hand motor functioning during simultaneous 10 Hz rTMS and cathodal tDCS. In contrast, simultaneous anodal tDCS increased the facilitatory effects of 10 Hz rTMS on corticomotor excitability and hand motor functions (Park et al., 2014). Hence, despite the contradictory results, these studies provide evidence that preconditioning rTMS with tDCS could enhance cortical plasticity and modulate rTMS effects. However, until now, studies combining these NIBS techniques have mainly targeted the motor cortex (e.g., Hasan et al., 2012; Park et al., 2014) and, surprisingly, studies employing the DLPFC as the target of stimulation in combined NIBS protocols are scarce. In an exploratory pilot study, Loo and colleagues (2009) investigated the neuropsychological and behavioral effects of preconditioning 10 Hz rTMS with tDCS over the DLPFC in 7 depressed patients. No evidence for an enhanced effect of combining 10 Hz rTMS with (anodal or cathodal) tDCS on mood and neuropsychological functioning was found (Loo et al., 2009). However, different NIBS combinations, number of sessions and stimulation parameters were used across patients which, in combination with

the small sample size and lack of sham tDCS comparison, make the interpretation of these results inconclusive.

Considering 1) the essential role of the DLPFC in the pathogenesis of several psychiatric disorders such as major depressive disorder (Koenigs and Grafman, 2009), and 2) the fact that the DLPFC is a commonly used stimulation site in tDCS and rTMS interventions for the treatment of depression (Baeken et al., 2013; Tortella et al., 2015), well-powered sham-controlled phase-I studies investigating the effects of these combined protocols on the DLPFC are required. Based on research of the motor cortex and the theory of functional targeting, one could expect that the effects of iTBS protocols applied to the DLPFC might be improved through the combination of iTBS with tDCS targeting the same neural network. Moreover, further understanding of underlying mechanisms associated with DLPFC stimulation using different NIBS protocols are essential for the optimization of therapeutic protocols.

Taking into account that the prefrontal brain regions are interconnected with the hypothalamus and limbic areas (Ledoux, 2000), and therefore are associated with differences in the psychology and physiology of the human stress response (Wheelock et al., 2016), we examine the effects of combined prefrontal tDCS-iTBS stimulation in the context of stress. Abnormal activity within the prefrontal-limbic circuit forms the basis of abnormal stress reactivity and is associated with negative affect and mood disorders (Mayberg, 2001). Moreover, anxiety and mood disorders are characterized by an elongated and hyperactive stress system (Pariante and Lightman, 2008), increased amygdala activity and decreased DLPFC functioning (Siegle et al. 2007; Wager et al., 2008). Interestingly, modulation of this prefrontal area through the use of NIBS techniques, such as tDCS or rTMS, results in a decreased psychophysiological stress response (e.g., Brunoni et al., 2013; Carnevali et al. 2020; Remue et al., 2016; Pulopulos et al., 2020), which might be one of the underlying mechanisms to the antidepressant effects of NIBS (Cirillo et al., 2017; Czéh et al., 2002). Indeed, in a recent study, Pulopulos and colleagues found that high frequency (HF; i.e., 20 Hz) rTMS over the left DLPFC influences physiological stress reactivity during the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) in a group of young healthy females. Specifically,

participants in the active HF rTMS condition showed lower stress-induced cortisol responses compared to participants in the sham condition (Pulopulos et al., 2020). In another study, Remue and colleagues (2016) found that healthy females who received active 20 Hz rTMS over the left DLPFC before being stressed using a negative feedback paradigm, showed a reduced physiological stress response, as indicated by an increase in heart rate variability (HRV), in comparison to participants who received sham HF rTMS. Similar HRV results were observed by Carnevali et al. (2020), using bifrontal tDCS before the TSST in a sample of young healthy males. Remarkably, whereas HRV typically shows a significant decrease in response to stress (e.g., Delaney and Brodie, 2000; Pieper et al., 2007; Pulopulos et al., 2020), these studies report a small decrease (Carnevali et al., 2020) or even an increase (Remue et al., 2016) in HRV during the confrontation with the stressor following bifrontal tDCS and active HF rTMS, respectively. Hence, NIBS techniques are effective in modulating activity of the autonomic nervous system, a key component in the physiological stress response (Iseger et al., 2020a; Makovac et al., 2017). Moreover, a recent study showed that heart rate and its variability might serve as a proxy of the direct effects of TMS over specific brain regions (Iseger et al., 2020b). Interestingly, in their systematic review, Schestatsky and colleagues (2013) suggested the combined application of TBS and tDCS as a promising tool to enhance the modulating of autonomic functioning. Hence, examining the effects of the combination of tDCS and iTBS on psychophysiological stress responses will provide insight into the effects of this protocol on physiological processes known to play an important role in stress-related disorders, such as major depression. Moreover, it could offer critical information about the safety and feasibility of this combination when used to target the DLPFC to improve therapeutic interventions.

The aim of this proof-of-concept study was to investigate the effects of the concurrent application of active iTBS with (active or sham) bifrontal tDCS applied to the DLPFC in an adequately powered study with healthy participants. Given that studies of the motor cortex describe different results for different types of tDCS stimulation (anodal versus cathodal; e.g., Hasan et al., 2012; Park et al., 2014) and, synaptic plasticity is suggested to occur via

coactivation of the cortical network targeted by the combined NIBS protocol (e.g., Jackson et al., 2016), we evaluated the effects of concurrent bifrontal tDCS and left prefrontal iTBS. Both NIBS techniques are commonly used in clinical trials with stress-related disorders such as major depression (e.g., Brunoni et al., 2014; Duprat et al., 2016). Moreover, bifrontal tDCS is considered advantageous to setups employing an extracephalic or supraorbital reference electrode because it simultaneously increases left and decreases right DLPFC activity thereby modulating the (medial) prefrontal network which is highly relevant for affective functioning (Banks et al., 2007; Brunoni et al., 2014). Furthermore, the effects of left prefrontal iTBS are ought to spread beyond the stimulation site via functional and/or structural connections to its associated hubs within the prefrontal-limbic circuit (Klooster et al., 2019; Klooster et al., 2020; Tang et al., 2019). The rationale for our study was that combined tDCS-iTBS stimulation would result in a stronger activation of the prefrontal network thereby maximizing the effects of iTBS over the DLPFC on the psychophysiological stress response. Taking into consideration the large interindividual differences and small effects sizes reported in past research evaluating the effects of NIBS on the psychophysiological stress response (e.g., Carnevali et al., 2020; De Witte et al., 2020; Pulopulos et al., 2019, 2020), we evaluated the additional effect of iTBS on stress in a within-subjects crossover design with a large study sample. Specifically, in order to investigate whether tDCS-iTBS would be more effective than iTBS alone, all participants performed two stimulation protocols (counterbalanced order, with one week in between): (1) bifrontal tDCS + active iTBS and (2) sham tDCS + active iTBS. A psychosocial stress task was used to activate the stress system after stimulation. Heart rate (HR), heart rate variability (HRV), blood pressure (BP) and electrodermal activity (EDA) were assessed as reliable, objective physiological measures of stress. EDA indexes the response of the sympathetic nervous system (Boucsein, 2012), whereas HRV reflects parasympathetic control (Thayer, 2006; Thayer et al., 2012) and, HR and BP are regulated by both sympathetic and parasympathetic pathways (Vrijlkotte et al., 2000; Wheelock et al., 2016). Self-report questionnaires were used as subjective measures of stress. Considering that the concurrent application of bifrontal tDCS and left prefrontal iTBS would result in a stronger activation of

the prefrontal network (i.e. coactivation of the DLPFC and its connected networks), we hypothesized that the combination of bifrontal tDCS and active iTBS (i.e., *bifrontal*tDCS+iTBS) would result in larger effects on the stress-induced psychophysiological response compared to the effects of sham tDCS and active iTBS (i.e., *sham*tDCS+iTBS). Specifically, after *bifrontal*tDCS+iTBS, compared to the *sham*tDCS+iTBS protocol, we expected to find a lower physiological (lower heart rate, blood pressure and electrodermal activity and higher heart rate variability) and psychological (e.g., lower perceived stress and negative affect) stress response during and following exposure to the stressor.

2. Methods

2.1 Study sample

Seventy-four healthy participants between 18 and 45 years old were recruited via flyers that were spread across the faculties of the Ghent University and social media platforms. All candidates were screened for past or current psychiatric diagnoses based on the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) and the International Classification of Diseases (ICD-10) using the semi-structured Mini International Neuropsychiatric Interview (MINI screening version 7.0.2; Sheehan, 2016). The Beck Depression Inventory-II (BDI-II) was used to check for depressive symptoms in the past two weeks (Beck et al., 1996; Dutch translation by Van der Does, 2002). For an overview of the selection criteria, we refer to the Supplementary Material. All participants gave written informed consent. The study is in accordance with the Declaration of Helsinki (2018) and approved by the Ethical Committee of the Ghent University Hospital.

2.2 Neurostimulation

In this within-subjects crossover design, all participants followed the same procedure in which they had two sessions of (bifrontal or sham) tDCS combined with active iTBS with one week in between to avoid carry-over effects. Depending on the session, the application of

tDCS was bifrontal tDCS (i.e., bifrontalDCS+iTBS condition) or sham tDCS (i.e., sham tDCS+iTBS condition). The order of the sessions was counterbalanced across participants. Participants were naïve to the stimulation protocol and were blindfolded and asked to wear earplugs during the stimulation sessions (Baeken et al., 2013, Duprat et al., 2016). Given that the success rate of correctly guessing the stimulation condition of each of the two sessions (33.82%) was not higher than chance level (50%), $p = 0.010$, 95% CI [0.23; 0.46], the blinding of the stimulation conditions was considered successful.

At the start of the first session, the individual resting motor threshold (rMT) of each participant was determined using motor evoked potentials. rMT was operationalized as the minimum TMS intensity necessary to yield a motor evoked potential in the right abductor pollicis brevis muscle that surpasses a peak-to-peak amplitude of 50 μ V in 5 out of 10 successive attempts (Rothwell et al., 1999). In all stimulation protocols, the DLPFC was targeted and localized using the Beam F3 localization system (Beam et al., 2009; Mir-Moghtadaei et al., 2015) to acquire optimal electrode and coil positioning. tDCS was applied using a Soterix mini-CT tDCS device (model 1601, Soterix Medical Inc., NY, United States) that allows automated double-blinding of tDCS conditions. Anode and cathode were respectively placed over F3 and F4 (Brodmann areas 8/9, Herwig et al., 2003), corresponding to the left and right DLPC, resulting in a bilateral DLPFC configuration. For iTBS, the stimulation coil was fixed over the left DLPFC. During the stimulation, participants were asked to keep their head as still as possible. Moreover, the experimenter remained near to ensure that participants did not move their head during the iTBS pulse delivery.

Participants received 20 minutes of bifrontal or sham tDCS. During bifrontal tDCS, a current flow of 2 mA was delivered through carbon rubber electrodes of 4.5x4.5 centimeters that were adhered to the head using electrical conductance paste (i.e., Ten20 paste, Weaver and Co., USA, Woods et al., 2016). During sham tDCS, participants were expected to have similar sensations as during real tDCS (e.g., itchy or tingling sensation at the stimulation site), but stimulation was too brief to cause any after-effects (Woods et al., 2016). Specifically,

during the first and last minute of sham tDCS stimulation, there was a ramp-up of 30 seconds until the current intensity reached 2 mA followed by a ramp-down of 30 seconds. During the last 7 minutes of the tDCS protocol, iTBS was applied using a figure-eight shaped coil (Magstim 70 mm double air film coil) connected to the Magstim Rapid² Plus¹ magnetic stimulator (Magstim Company Limited, Wales, UK). For each session, the following parameters were used: 54 cycles including 10 bursts of 3 pulses each - at a frequency of 50 Hz and burst frequency 5 Hz - with a train duration of 2 seconds and with a cycling period of 8 seconds, resulting in a total of 1620 pulses with a power output of 110% of the resting motor threshold. The tDCS and iTBS parameters used in this study are based on the parameters that we used to treat depressed patients (Brunoni et al., 2014, Duprat et al., 2016). Given that the iTBS and tDCS protocols differ in duration (i.e., 7 minutes iTBS versus 20 minutes tDCS), we decided to apply iTBS during the last 7 minutes before the end of the tDCS protocol. As such, tDCS was applied alone for 13 minutes thereby modulating the initial activation state of the stimulated region/network (Bikson and Rahman, 2013) before applying iTBS concomitantly which allowed for an induction of synaptic plasticity via coactivation of the same targeted network (Jackson et al., 2016).

Side effects from the combined stimulation protocol were assessed at the end of each session using a semi-structured interview. All reported experiences were classified in 7 categories: burning sensations under the electrodes, tingling sensations on the scalp, lightheadedness, facial muscle contractions, headache, non-specific discomfort and pain associated with the stimulation.

2.3 The Stress Task

To induce a psychological and physiological stress response in the participants, the Maastricht Acute Stress Test (MAST; Smeets et al., 2012) was used. In the MAST, aspects from the two most used stress paradigms are combined, namely social-evaluative threat (e.g., negative feedback) and uncontrollability (e.g., unknown duration of the task) from the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) and physical stress from the Cold Pressor

Task (CPT; Lovallo, 1975). In general, the MAST is an effective stress induction paradigm (Shilton et al., 2017) which has been shown to repeatedly generate a robust psychological and physiological stress response, without participants showing any habituation or sensitization in response to the paradigm (Quaedflieg et al., 2017). For the specific procedure and set-up of the MAST, we refer to the Supplementary Material.

2.4 Psychophysiological assessments

2.4.1 Physiological measures

Heart rate and electrodermal activity were acquired with the Biopac MP150 system and the Biopac Acqknowledge software 4.3 (Biopac Systems Inc., USA). More specifically, the Biopac ECG100C Electrocardiogram and EDA100C-MRI amplifier were used, at a sample rate of 1000 Hz, in order to measure cardiac activity and electrodermal activity (i.e. skin conductance levels), respectively. From the electrocardiogram, mean heart rate (HR) and heart rate variability (HRV) were derived. HRV was computed using the Root Mean Square of Successive Differences (RMSSD), a vagally-mediated, time-domain index of HRV known to be less susceptible to movement and respiration artefacts than other HRV indices (Laborde et al., 2017; Shaffer and Ginsberg, 2017). Systolic and diastolic blood pressure (i.e., SBP and DBP) was assessed using a validated oscillometric device (OMRON M6 Comfort; Belghazi et al., 2007). For details on the set-up, recording parameters and pre-processing of the physiological measures data, we refer to the Supplementary Material.

2.4.2 Psychological measures

Participants repeatedly rated their affective states on Visual Analogue Scales (VAS) to detect changes in perceived stress and negative affect throughout the sessions. Each VAS consisted of a 100 millimeters straight line with captions at both ends referring to the extremes of a subjective experience ("I do not experience this at all", "I experience this very much"). On each scale, participants had to mark the point of the line that corresponded to their affect state at that time. VAS measuring happiness, tension and worry were combined to get a composite

negative affect score (happy scales were reversed, McCormack et al., 1988). Higher scores indicate higher levels of perceived stress or negative affect.

2.5 Procedure

All experimental sessions took place in a well-controlled laboratory environment in the Ghent University Hospital. Participants were asked to sleep sufficiently, restrain from intense physical activity and alcohol 24 hours prior to the session and not eat or consume any caffeinated beverages 2 hours before coming to the hospital. Participants were seated in a TMS chair in high Fowler's position during the entire session. The equipment for the physiological measures was connected and afterward participants were asked to remain seated and relax for 10 minutes to habituate to the laboratory. Subsequently, a first baseline measurement was assessed. After the habituation, the stimulation equipment was set-up and stimulation was applied. Depending on the stimulation condition, participants received 20 minutes of bifrontal or sham tDCS. During the last 7 minutes of tDCS, iTBS was applied. Afterward, participants were introduced to the MAST and received instructions via a PowerPoint presentation. The following 10 minutes, participants went through the acute stress phase of the MAST. After the stress task, there was a 10-minute recovery period. During the entire session (see Figure 1 for an overview), participants were asked to rate their affective state five times on Visual Analogue Scales (VAS) after which blood pressure measures were taken. At the end of each session, participants were questioned about possible side effects during or directly after the combined tDCS-iTBS protocol. At the end of the study, participants were debriefed about the purpose of the study and received a monetary compensation for participation.

2.6 Statistical Analyses

The required sample size for a low to moderate effect size ($f = 0.175$) was calculated with G*Power 3.1 software (Faul et al., 2009). We included two measurements, set alpha at 0.05 and power at 0.80, which resulted in a sample size of 67 participants. Considering

possible losses or dropouts (10%), we recruited 74 participants. However, 6 participants were removed from the analyses (see Supplementary Material for reasons of exclusion) resulting in a final study sample of 68 participants. Due to issues with the EDA electrodes, there were less participants for the EDA analyses ($n = 61$).

Linear mixed effects analyses were performed with R software version 3.6.1 (R Core Team, 2019) using the 'lme4' and 'lmerTest' packages (Bates, 2015; Kuznetsova et al., 2017). For blood pressure (i.e., SBP and DBP) and mood ratings (i.e., perceived stress and negative affect), a 2 (condition: sham tDCS+iTBS versus $\text{bifrontal tDCS+iTBS}$) by 5 (time: baseline, post stimulation, post instructions, post stress, post stress recovery) linear mixed model was fitted. A 2 (condition: sham tDCS+iTBS versus $\text{bifrontal tDCS+iTBS}$) by 6 (time: baseline, tDCS-only, tDCS+iTBS, stress instructions, stress reactivity, stress recovery) linear mixed model was used for the mean HR, HRV and EDA data. In all models, we included random intercepts for subjects and controlled for order. The natural logarithm ($\log$) was calculated for all physiological measures and square-root (sqrt) transformations were applied to the mood ratings in order to meet the model's assumptions of normality of the residual distributions. All p -values were provided using the Kenward-Roger degrees of freedom approximation and Tukey-adjusted p -values were used for all post-hoc analyses. The significance level was set at $\alpha = 0.05$. Effect sizes were described using the partial eta squared (i.e., η_p^2) and Cohen's d_{av} for F-tests and t-tests, respectively (see also Lakens, 2013). Bayesian repeated measures analyses were performed to complement the linear mixed effects analyses (Quintana and Williams, 2018). All Bayesian analyses were performed using JASP version 0.10.2 (JASP team, 2019) with default prior scales. Bayes factors quantifying the evidence in favor of the null (BF_{01}) or the alternative (BF_{10}) hypotheses, with the first being the inverse of the latter, were reported and described according to the classification scheme used in JASP (Lee and Wagenmakers, 2014, adopted from Jeffreys, 1998). For a complete overview of the results from the linear mixed effects analyses and the Bayesian analyses, see Table 1 of the Supplementary Material.

3. Results

3.1 Sample characteristics.

An overview of the study sample characteristics can be found in table 1.

3.2 Systolic and Diastolic Blood Pressure

The linear mixed effects analyses showed no significant interaction between time and condition for SBP, $F(4,603) = 0.94$, $p = 0.440$, $\eta_p^2 = 0.01$, and DBP, $F(4,603) = 1.37$, $p = 0.244$, $\eta_p^2 = 0.01$. According to the Bayesian analyses, there was very strong evidence for the absence of an effect of the *bifrontal*tDCS+iTBS protocol compared to *sham*tDCS+iTBS on SBP. Specifically, the SBP data was 31.81 more likely to occur under the null hypothesis as compared to the alternative hypothesis. Furthermore, there was strong evidence for the absence of an interaction effect on DBP (interaction effect $BF_{01} = 16.39$).

Results of the linear mixed effects analyses revealed a significant main effect of time for both SBP, $F(4,603) = 111.41$, $p < 0.001$, $\eta_p^2 = 0.42$, and DBP, $F(4,603) = 109.05$, $p < 0.001$, $\eta_p^2 = 0.42$ (see Figure 2a,2b). After the exposure to the stress task, there were significant higher levels of both SBP, $t(603) = 17.78$, $p < 0.001$, $d_{av} = 1.13$, and DBP, $t(603) = 18.81$, $p < 0.001$, $d_{av} = 1.54$, compared to baseline. Following the 10-minute stress recovery period, all blood pressure values significantly decreased (SBP: $t(603) = 15.62$, $p < 0.001$, $d_{av} = 1.01$; DBP: $t(603) = 12.98$, $p < 0.001$, $d_{av} = 1.11$; for an overview of all post hoc comparisons between the different timepoints, we refer to Tables 2 and 3 of the Supplementary Material). For both SBP and DBP, the results showed no effects of order ($F_s < 0.12$, $p_s > 0.735$), nor condition ($F_s < 0.26$, $p_s > 0.617$).

3.3 Heart Rate and Heart Rate Variability

In contrast to the a priori hypotheses, the linear mixed effects analyses showed no interaction between time and condition for both mean heart rate (HR), $F(5,737) = 0.80$, $p =$

0.550, $\eta_p^2 = 0.01$, and HRV, $F(5,737) = 0.27$, $p = 0.931$, $\eta_p^2 = 0.002$. This was corroborated by Bayesian analyses, showing very strong to extreme evidence for the absence of an effect of the combined stimulation protocol on mean HR (interaction effect $BF_{01} = 75.94$) and HRV (interaction effect $BF_{01} = 211.57$).

Results of the linear mixed effect analyses showed a significant main effect of condition on mean HR, $F(1,737) = 9.14$, $p = 0.003$, $\eta_p^2 = 0.01$, with lower mean HR in the bifrontal tDCS+iTBS condition compared to the sham tDCS+iTBS, $t(737) = 3.02$, $p = 0.003$, $d_{av} = 0.10$. For HRV, results of the linear mixed model also revealed a significant main effect of condition, $F(1,737) = 7.65$, $p = 0.006$, $\eta_p^2 = 0.01$. Overall, HRV levels were higher in the bifrontal tDCS+iTBS condition compared to the sham tDCS+iTBS condition, $t(737) = 2.77$, $p = 0.006$, $d_{av} = 0.11$. In addition, there was a significant main effect of time for both mean HR, $F(5,737) = 132.44$, $p < 0.001$, $\eta_p^2 = 0.47$, and HRV, $F(5,737) = 40.23$, $p < 0.001$, $\eta_p^2 = 0.21$ (see figure 2c,2d). Both HR, $t(737) = 11.09$, $p < 0.001$, $d_{av} = 0.66$, and HRV, $t(737) = 6.49$, $p < 0.001$, $d_{av} = -0.44$, were significantly higher during the MAST as compared to baseline. During stress recovery, as compared to stress reactivity, there were significant lower levels of mean HR, $t(737) = 23.86$, $p < 0.001$, $d_{av} = 1.45$, and higher levels of HRV, $t(737) = 5.58$, $p < 0.001$, $d_{av} = 0.40$ (for an overview, see Tables 4 and 5 of the Supplementary Material). No effects of order were found, $F_s < 0.63$, $p_s > 0.429$.

3.4 Electrodermal activity

The linear mixed effects analyses showed no significant interaction between time and condition, $F(5,660) = 0.39$, $p = 0.857$, $\eta_p^2 = 0.003$. Results of the Bayesian analyses showed extreme support for the absence of an interaction effect between time and condition, with the EDA data being 132.37 times more likely under the null hypothesis than the alternative hypothesis ($BF_{01} = 132.37$).

The linear mixed effects analyses revealed a significant main effect of condition, $F(1,660) = 5.55$, $p = 0.019$, $\eta_p^2 = 0.01$, with an overall higher EDA in the sham tDCS+iTBS

condition compared to the _{bifrontal}tDCS+iTBS condition, $t(658) = 2.634$, $p = 0.009$, $d_{av} = 0.08$. There was a significant main effect of time, $F(5,660) = 152.21$, $p < 0.001$, $\eta_p^2 = 0.54$ (see Figure 2e). EDA was significantly increased during the exposure to the stress task as compared to baseline, $t(660) = 24.77$, $p < .001$, $d_{av} = 1.51$. During stress recovery, EDA was significantly lower than the EDA during the stress task, $t(660) = 3.87$, $p = 0.002$, $d_{av} = 0.30$ (see also Table 6 of the Supplementary Material). Results showed no effect of order, $F(1,59) = 1.08$, $p = 0.303$, $\eta_p^2 = 0.02$.

3.5 Psychological measures

In contrast to the a priori hypotheses, the mixed effects analyses revealed no evidence for an interaction effect between time and stimulation condition for both perceived stress, $F(4,603) = 1.99$, $p = 0.095$, $\eta_p^2 = 0.01$, and negative affect, $F(4,603) = 1.09$, $p = 0.358$, $\eta_p^2 = 0.01$. Moreover, the Bayesian analyses showed anecdotal to strong evidence in favor of the null hypothesis for perceived stress (interaction effect $BF_{01} = 1.70$) and negative affect (interaction effect $BF_{01} = 23.36$), respectively.

Results of the linear mixed effect analyses showed significant main effects of time for perceived stress (see Figure 2f), $F(4,603) = 43.05$, $p < 0.001$, $\eta_p^2 = 0.22$, and negative affect (see Figure 2g), $F(4,603) = 63.95$, $p < 0.001$, $\eta_p^2 = 0.30$. The level of perceived stress peaked after the exposure to the stress task, $t(603) = 9.36$, $p < .001$, $d_{av} = 0.87$. Similarly, negative affect levels were significantly higher after the stress task, $t(603) = 11.66$, $p < 0.001$, $d_{av} = 1.00$. During stress recovery, the levels of perceived stress, $t(603) = 11.25$, $p < 0.001$, $d_{av} = 1.01$, and negative affect, $t(603) = 14.41$, $p < 0.001$, $d_{av} = 1.13$, were significantly lower than after the stress task (we refer to Tables 7 and 8 of the Supplementary Material for an overview of all time contrasts). For both psychological measures, there were no significant effects of condition or order ($F_s < 1.38$, $p_s > 0.211$).

3.6 Side effects

No seizures or extreme side effects were observed during or immediately after the combined tDCS-iTBS protocol. Participants reported more tingling sensations on the scalp and, discomfort and pain associated with the stimulation following the *bifrontal*tDCS+iTBS protocol as compared to the *sham*tDCS+iTBS stimulation (see table 2).

4. Discussion

The aim of this proof-of-concept within-subjects study in healthy volunteers was to investigate whether the effects of iTBS applied to the left DLPFC on psychophysiological measures of stress reactivity and recovery could be enhanced by the concurrent application of bifrontal tDCS. We found strong time effects and the MAST elicited robust, autonomic responses following both stimulation conditions. In line with previous studies, there was a significant increase in physiological (i.e., heart rate, blood pressure and skin conductance level) and psychological (i.e., perceived stress and negative affect) stress indices in response to the MAST (Shilton et al., 2017, Smeets et al., 2012). However, contrary to our hypotheses, combining iTBS with tDCS did not attenuate the psychophysiological stress response as no differences were found between the two stimulation protocols during stress reactivity and recovery. These findings were corroborated by Bayesian analyses that showed strong to extreme evidence for the lack of an additional effect of combining iTBS with tDCS on physiological measures of stress reactivity and recovery.

In fact, the combined stimulation protocol (i.e., *bifrontal*tDCS+iTBS) was found to be more painful and subjects reported more tingling sensations on the scalp and feelings of discomfort compared to the *sham*tDCS+iTBS protocol. These results are in line with the findings of Loo and colleagues (2009) that also reported increased pain experiences in a combined protocol over the prefrontal cortex. No such findings were reported in any of the studies on the motor cortex (e.g., Hasan et al., 2012). However, these studies applied TBS for a relatively short period, with the total number of pulses varying between 100 to 900 pulses, and at lower stimulation frequencies (e.g., 1 Hz or 5 Hz rTMS). Overall, iTBS at higher intensities (e.g., 110

or 120%) has been found to be more painful compared to rTMS protocols using lower parameters (Blumberger et al., 2018), even though *bifrontal*tDCS+iTBS was reported more painful as *sham*tDCS+iTBS. Also, as suggested by Loo and colleagues (2009), tDCS might cause a depolarization of the membrane potential of the somatosensory nerve fibers surrounding the electrodes which may lead to more pain associated with the stimulation during the concurrent tDCS-iTBS protocol.

Several studies have demonstrated the therapeutic effects of tDCS and iTBS independently (e.g. Brunoni et al., 2012; Duprat et al., 2016) and, the ability of each of these NIBS techniques to modulate autonomic nervous activity both at rest as in the context of stress (e.g., Brunoni et al., 2013; Carnevali et al., 2020; Remue et al., 2016; Pulpulos et al., 2020). However, the results of this well-powered proof-of-concept study suggest that the combination of tDCS and iTBS applied to the DLPFC does not necessarily lead to an additional effect, at least not with the stimulation parameters used in this study. Hence, our results are restricted to the particular protocol employed and, although the same parameters have shown to be effective in modulating prefrontal functioning for each of the NIBS techniques independently (e.g., Brunoni et al., 2012; Carnevali et al., 2020; Duprat et al., 2016), it is possible that these NIBS parameters, when used in combination, lead to homeostatic mechanisms that eliminate the possible advantage of tDCS. Indeed, there is a physiological rationale that other NIBS parameters, such as cathodal tDCS+iTBS (e.g., Hasan et al., 2012), could have yielded different results. However, we cannot be certain that the combination of iTBS and tDCS over the prefrontal cortex would induce similar effects as described in the literature on the motor cortex. Also, other forms of NIBS combinations can yield potential to enhance rTMS effects and need to be explored. Hence, further research is warranted. Nonetheless, our study was adequately powered and showed that the combination of tDCS and iTBS of the DLPFC did not modulate psychological and physiological stress responses above iTBS alone in the presented sample with the employed set-up and stimulation parameters.

Although this proof-of-concept study has some major strengths, such as the use of a large sample size and a within-subjects cross-over study design, some limitations should be discussed. An important restriction to the study is the lack of $\text{bifrontal tDCS} + \text{sham iTBS}$ condition and $\text{sham tDCS} + \text{sham iTBS}$ condition to evaluate the effectiveness of respectively tDCS and iTBS alone on psychophysiological measures of stress reactivity and recovery in the presented sample. Although several studies have reported the standalone effects of bifrontal tDCS on stress-related outcomes (e.g., Carnevali et al., 2020), studies evaluating the effects of iTBS alone on the psychophysiological stress response are scarce. Interestingly, two recent studies from our group demonstrated that, when taking interindividual differences into account, iTBS influences cortisol stress recovery after a psychosocial stressor (De Witte et al., 2020; Pulopulos et al., 2019). However, given that the participants in these studies were exposed to a stressor before stimulation, the stimulation effects were solely assessed during stress recovery. Hence, future studies evaluating the effects of iTBS on stress reactivity are warranted. Nonetheless, previous research has demonstrated the positive effects of HF rTMS on physiological stress reactivity (e.g., Remue et al., 2016). Remarkably, in line with these studies (e.g. Remue et al., 2016), the results of the current study indicated higher levels of HRV during the MAST as compared to baseline (whereas HRV typically decreases in response to the stressor e.g., Delaney and Brodie, 2000; Pieper et al., 2007; Pulopulos et al., 2020). Indeed, past research has demonstrated that NIBS techniques, such as rTMS and tDCS, are able to increase HRV (Iseger et al., 2020a; Makovac et al., 2017), especially when targeting the prefrontal cortex, a key region in the regulation of HRV (Thayer et al., 2012). It is suggested that NIBS of the prefrontal cortex might lead to more prefrontal inhibition of amygdalar activity and activity in sympatho-excitatory neural circuits when exposed to a stressor, resulting in an increase in HRV (Thayer et al., 2012). Since HRV is considered an index of how adaptive the autonomic nervous system responds to changing environmental demands, increased levels of HRV during the MAST might suggest successful stress regulation induced by the iTBS (Thayer, 2006; Thayer et al., 2012). However, although these

findings are in line with the literature, given that there was no sham iTBS condition nor baseline stress measure in the current study, this increase in HRV during the MAST should be interpreted with caution and no conclusive inferences can be made. Hence, future studies are recommended to employ a full factorial design to evaluate the effects of each of the techniques alone and in combination on prefrontal cortex functioning. Another limitation of the current study is the lack of a direct marker of brain activity in response to the combined protocol. Whereas TMS studies on the motor cortex use motor-evoked potential as an objective measure of the effects of stimulation, the stress-related outcomes used in this study do provide a direct quantification of the effectiveness of combined stimulation over the DLPFC. Nevertheless, stress and emotion regulation are well known processes directly related to prefrontal cortex functioning. However, future studies of combined NIBS of the DLPFC using functional imaging techniques, such as electroencephalography (EEG) or functional magnetic resonance imaging (fMRI), may provide more sensitive measures to detect changes in neuronal responses to the stimulation combination. Moreover, more direct markers of neural activity (instead of indirect markers such as psychophysiological measures of stress reactivity and recovery) may provide crucial insights into the effects of combined NIBS within the relevant prefrontal circuits. Specifically, using functional imaging, it could have been evaluated whether there are simply no additive effects of combining iTBS with tDCS on psychophysiological stress responses or whether the obtained null effects in this study result from an adequate modulation of the relevant circuits in both stimulation conditions. Hence, further research employing functional imaging approaches are warranted. However, of important note, given that the results of this proof-of-concept study indicate that combined tDCS-iTBS over the DLPFC, with the employed stimulation parameters, may cause pain and feelings of discomfort, researchers may want to consider other stimulation parameters or other forms of NIBS combinations that can yield potential to enhance rTMS effects but cause less pain and discomfort, especially when choosing a functional imaging approach.

To conclude, with the stimulation parameters used in this study, there is strong evidence for the absence of an additional effect of combining iTBS with tDCS on psychophysiological measures of stress reactivity and recovery in healthy subjects. Moreover, the concurrent application of bifrontal tDCS and active iTBS resulted in more feelings of discomfort and pain. Future studies should be aware of these increased side effects associated with the combined tDCS-iTBS protocol and should explore other stimulation parameters and/or other additive approaches, preferably employing a full factorial design and more direct markers of neuronal responses to the combined NIBS protocol.

Declaration of competing interest

None of the authors have potential conflicts of interest to be disclosed.

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Legends

Figure 1. Experimental procedure. During the stimulation period, sham or bifrontal tDCS was administered for 20 minutes. During the last 7 minutes of the tDCS protocol, active iTBS was applied. Abbrev.: BDI-II, Beck-Depression Inventory-II; BP, blood pressure; ECG, electrocardiogram; EDA, electrodermal activity; iTBS, intermittent theta burst stimulation; MAST, Maastricht acute stress test; tDCS, transcranial direct current stimulation; VAS, visual analogue scales.

Figure 2. The average values across participants of the different psychophysiological measures throughout the experimental session for the _{sham}tDCS+iTBS condition and _{bifrontal}tDCS+iTBS condition. Error bars depict 95% confidence intervals. Abbrev.: DBP, diastolic blood pressure; HR, heart rate; HRV, heart rate variability; iTBS, intermittent theta burst stimulation; mmHg, millimetres of mercury; ms, milliseconds; RMSSD, root mean square of successive differences; SBP, systolic blood pressure; tDCS, transcranial direct current stimulation; VAS, visual analogue scales; μ S, microSiemens.

Tables

Table 1. Demographic characteristics of the study sample.

	Total (<i>n</i> = 68)	Females (<i>n</i> = 42)	Males (<i>n</i> = 26)
Age	21.87 (3.50)	21.45 (3.30)	22.54 (3.69)
BDI-II	5.34 (4.60)	5.59 (4.68)	4.92 (4.44)
SES	5.40 (1.48)	5.40 (1.33)	5.38(1.69)
Smokers	6	1	5
BMI	22.54 (2.64)	22.29 (2.87)	22.93 (2.16)

Mean (SD, i.e. standard deviation) for age, scores on the Beck Depression Inventory (BDI-II), Subjective Socio-economic Status (SES ; measured using the nine-rung 'social ladder', cf., Adler et al., 2000) and Body Mass Index (BMI). Smokers are presented in absolute numbers.

Table 2. Reported side effects.

	sham tDCS-iTBS	bifrontal tDCS-iTBS	Statistics
Burning sensations	13.2%	16.2%	$\chi^2(1,68) = 0.2, p = 0.809$
Tingling sensations	52.9%	72.1%	$\chi^2(1,68) = 5.3, p = 0.033$
Lightheadedness	4.4%	5.9%	$\chi^2(1,68) = 0.2, p = 0.698$
Facial muscle contractions	16.2%	14.7%	$\chi^2(1,68) = 0.6, p = 0.812$
Headache	2.9%	4.4%	$\chi^2(1,68) = 0.2, p = 0.649$
Discomfort	22.1%	39.7%	$\chi^2(1,68) = 4.9, p = 0.041$
Pain	2.9%	14.7%	$\chi^2(1,68) = 5.8, p = 0.031$

Percentage of subjects reporting above-mentioned side effects. Chi-squared statistics were used to compare the number of reported side-effects between the two stimulation protocols. Abbrev.: tDCS, transcranial direct current stimulation; iTBS, intermittent theta burst stimulation.