The Effect of a Single HF-rTMS Session Over the Left DLPFC on the Physiological Stress Response as Measured by Heart Rate Variability

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Objectives: Previous research has demonstrated that prefrontal activity is related to control over stress responses. However, the causal mechanisms are not well understood. In this study we investigated the possible influence of brain stimulation on the physiological stress response system. Because an increased stress response is known to precipitate psychiatric disorders, further inquiry can have important clinical implications. Methods: In 38 healthy, right-handed female participants, we examined the effects of a single sham-controlled high-frequency (HF) repetitive Transcranial Magnetic Stimulation (rTMS) session over the left ($n = 19$) and right ($n = 19$) dorsolateral prefrontal cortex (DLPFC) on the autonomic nervous system stress response, as measured by heart rate variability (HRV). Stress was transiently induced through evaluative negative feedbacks. Results: Although the induction procedure was efficient in increasing self-reported distress in all groups and conditions, only after real HF-rTMS over the left DLPFC the physiological stress response was diminished, as indicated by a significant increase in HRV. No effects were found in the sham or right side stimulation condition. Conclusions: These findings demonstrate that increasing brain activity by HF-rTMS over the left DLPFC can help attenuating physiological stress reactions. Results are indicative of the positive effects of rTMS on stress resilience and underscore the possible benefit of HF-rTMS as a transdiagnostic intervention. Finally, the results also show that effects only occur when stimulating the left DLPFC, which is in line with the therapeutic effects of HF-rTMS in affective disorders.

Keywords: repetitive transcranial magnetic stimulation, heart rate variability, stress reactivity, dorsolateral prefrontal cortex, lateralization

Stressful situations are part of everyday life, but only a select population of individuals develops stress-related pathologies. Although stress is a broad construct, in this study we define it as a state of apprehension, accompanied by negative affect and autonomic arousal, close to the concept of state anxiety (Spielberger et al., 1983). According to the reactivity hypothesis, frequent elevated physiological responses during stressful events lead to changes in physiological balance, triggering several pathogenic pathways (Pieper, Brosschot, van der Leeden, & Thayer, 2007). More specifically, psychological stress confers risk for many cardiovascular diseases, including hypertension and myocardial infarction (Figueredo, 2009), and stressors can suppress cardiac parasympathetic nervous system activity in most individuals (Gianaros et al., 2005). Previous research has shown that confrontation with stressful situations is known to precipitate psychiatric disorders such as major depressive disorder (MDD; Wood, Walker, Valentino, & Bhatnagar, 2010). In Waugh and Koster (2015) the authors described resilience as a dynamic process that may be a grant from the Scientific Fund W. Gepts UZ-Brussels (Awarded to Chris Baeken). Preparation of this paper was also supported by Grant BOF10/GOA/014 for a Concerted Research Action of Ghent University (awarded to Rudi De Raedt) and an ERC Starting Grant (#200758). This work was also supported by the Ghent University Multidisciplinary Research Partnership “The Integrative Neuroscience of Behavioral Control.”

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deficient in people in remission from depression, rather than as a static personal quality that is unattainable to people who have experienced psychopathology. Moreover, depression is associated with multiple indicators of physiological dysregulation, including potentially diminished levels of cardiac vagal control (Rottenberg, Cliff, Bolden, & Salomon, 2007), affecting stress-related responses to environmental experiences (Disner, Beevers, Haigh, & Beck, 2011). Furthermore, the more recent concept of “allostatic load” (McEwen, 2007) posits that (a) the brain actively maintains homeostasis through the activation of prefrontal brain areas, autonomic, and endocrine systems; and (b) chronic load on these systems by persistent threat has deleterious effects on the brain and body, contributing to a variety of health problems (Kiecolt-Glaser & Glaser, 2002).

Anatomically, sympathetic and parasympathetic branches of the autonomic nervous system dually innervate the myocardium. Functionally, parasympathetic inputs provide constant, although fluctuating, inhibitory control of heart rate (HR) via direct innervation of the heart by the vagus nerve (for a more in depth discussion on cardiac vagal control, see Cyranowski et al., 2011). Stressor-induced suppression of cardiac parasympathetic activity (Gianaros et al., 2005) has been documented in a growing number of studies investigating heart rate variability (HRV) as an indirect measure of parasympathetic (vagal) control over time-related variations in heart rate (e.g., Berntson et al., 1997). HRV is a noninvasive, simple, and frequently used measure of autonomic influences on heart rate. Evidence indicates that HRV indices of sympathetic and parasympathetic activation pattern reflect biomarkers not only for cardiovascular health, but also for complex patterns of brain activations (e.g., Sloan, Korten, & Myers, 1991). HRV has also been used as an objective descriptor of individual differences in regulating emotional conditions or cognitive functions (e.g., Ahs, Sollers, Furmark, Fredrikson, & Thayer, 2009; Kemp et al., 2010; Thayer, Hansen, Saus-Rose, & Johnsen, 2009). HRV thus provides information on the nervous system’s ability to organize homeostatic responses in adaptation to the physiological and psychological requirements of the present situation (e.g., Appelhans & Luecken, 2008; Segerstrom & Nes, 2007). High HRV has been associated with greater behavioral adaptability and is related to adaptive recovery from stress (Thayer & Lane, 2009). Moreover, decreases of HRV have been related to worry (Pieper et al., 2007). Hence, we focus on HRV as a measure of stress responsiveness, which is one of the important physiological parameters in the context of the interplay between physiological and psychological phenomena (Wheat & Larkin, 2010). Interestingly, depression is associated with elevated heart rate and reduced HRV, which are known risk factors for cardiac morbidity and mortality that may explain the increased risk associated with depression (Carney, Freedland, & Veith, 2005; Thayer & Lane, 2007; Tsuji et al., 1996). Gianaros and colleagues (2004) emphasize that cortical and subcortical brain systems regulating cardiac autonomic activity during behavior have been extensively investigated in nonhuman animals (see reviews by Loewy & Spyer, 1990; Neafsey et al., 1993), but they questioned whether similar brain systems regulate behaviorally integrated cardiac autonomic activity in humans. This is an important question because the brain’s regulation of cardiac autonomic activity is claimed to influence a range of behavioral processes such as reactions to stressors (Lovallo & Gerin, 2003). A wealth of nonhuman animal literature supports a connection between medial PFC (mPFC) and autonomic control of the heart (e.g., Bandler, Keay, Floyd, & Price, 2000; Barbas, Saha, Rempel-Clower, Ghashghaei, 2003; Devinsky, Morrell, & Vogt, 1995).

Interestingly, the ventral mPFC has been linked to higher-order contextual control over stress responses, providing a conceptual link with the cognitive generation and regulation of stress responses in humans (Wager et al., 2009). In human imaging studies, the dorsal cingulate/mPFC has been linked consistently with stress-induced increases in HR, blood pressure (e.g., Gianaros, Van Der Veen, & Jennings, 2004; Gianaros, Sheu, Matthews, Jennings, Manuck, & Hariri, 2008; Critchley et al., 2003) and cortisol (Eisenberger, Taylor, Gable, Hilmert, & Lieberman, 2007).

Thus, experimental manipulation is necessary to increase our insight in the possible causal relationship between brain functioning and the stress response. In contemporary brain research, repetitive transcranial magnetic stimulation (rTMS) has evolved as a well-established brain stimulation tool, becoming a mainstay of cognitive neuroscience in a variety of applications (for a review, see Guse, Falkai, & Wobrock, 2010). rTMS is a noninvasive method of neuronal depolarization of specific areas of the human brain that can alter cortical excitability (George et al., 2003). Low frequency (LF) TMS is defined by rates ≤1 Hz, and high-frequency (HF) by those ≥1 Hz (up to 30 or more Hz) (Rossi & Rossini, 2004). Low-frequency rTMS is capable of temporarily decreasing cortical excitability after stimulation, whereas high-frequency rTMS increases it (Post & Keck, 2001). It emerged as a new technology that holds promise for investigating the relationship between attentional control and emotion processing (Vanderhasselt, De Raedt, Leyman, & Baeken, 2009), insight into the pathophysiology of a variety of stress-related mental disorders (Akirav & Maroun, 2007). In addition, the effects of rTMS on neurophysiology is well understood (for an in depth discussion on neurostimulation and neurophysiological effects, see Hoogendam et al., 2010; Rossi et al., 2009; Sandrini et al., 2011).

The left DLPFC has been implicated in the modulation of negative emotions and is a cortical target for rTMS treatment of depression (Borkardt et al., 2008), but it remains unclear whether HF-rTMS over this area can affect stress responsiveness. To test causal hypotheses, neurostimulation techniques might be very valuable. Unfortunately, with the currently used superficial coils it is impossible to directly target the vmPFC using rTMS. Previous research on the hypothalamic-pituitary-adrenal (HPA) system has shown that stimulating the left DLPFC indirectly influences cortisol secretion. Here, as well as in animal studies (Keck, 2003), it can be concluded that the neurobiological effects following DLPFC stimulation were established via indirect multimodal pathways (e.g., Baeken et al., 2009, 2010; Baeken, Vanderhasselt et al., 2014), affecting implicated subcortical and vmPFC structures.

In the present study, we aimed to go beyond previous—although limited—stress response and brain stimulation research, by using a single sham controlled HF-rTMS session over the left and right DLPFC as a method to temporally influence stress responsiveness of healthy people. Although treatment protocols for psychiatric disorders such as depression typically involve HF-rTMS over the left DLPFC (De Raedt, Vanderhasselt, & Baeken, 2015), given the absence of conclusive data on lateralization regarding the physiological stress response, we decided to include right and left stimulation groups. To induce stress, we used a performance feedback...
task, that is, the critical feedback task (adapted from Rossi & Pourtois, 2012a), which makes use of negative feedbacks referring to participants’ task-performance. Moreover, difficult cognitive tasks have been shown in numerous studies to elicit cardio-acceleration (reduced HRV), which is often mediated by decreased parasympathetic cardiac activity (e.g., Bernston et al., 1994). Hence, we assessed whether the stress response after negative feedback can be decreased by means of a single HF-rTMS session over the DLPFC. We hypothesized that, compared to sham stimulation, participants would exhibit higher HRV, as sign of lower stress response, immediately after a single HF-rTMS session over the DLPFC. In line with the observation that decreased left DLPFC activity has been implicated in the modulation of negative emotions, and the beneficial effect of left-sided stimulation in depression, we mainly anticipated effects after real stimulation of the left DLPFC.

Methods and Materials

Participants

The left side stimulation sample consisted of 23 healthy, right-handed females recruited among undergraduates. This population may be particularly relevant because stress-related disorders such as depression occur twice as often in women as in men (Kessler, McGonagle, Swartz, Blazer, & Nelson, 1993). We excluded one participant based on a high score on the BDI-II (>30), and three participants were not included because of technical problems. The final left side stimulation sample had a mean age of 21.84 (SD = 2.95). The right side stimulation sample consisted of 19 healthy, right-handed females with a mean age of 21.74 (SD = 1.76). For both groups, current and past psychiatric (both Axis I and Axis II) disorders were excluded using the Mini-International Neuropsychiatric Interview (MINI; Sheehan et al., 1998). No drugs were allowed, except birth-control pills. The ethics committee of the University Hospital of the Vrije Universiteit Brussel approved the study. All participants gave written informed consent and were financially compensated. A part of the participants in this study also participated in a larger project investigating the influence of HF-rTMS on different neurocognitive (neuroimaging) and genetic markers, and on the hypothalamic pituitary adrenal axis (Baeken, Vanderhasselt et al., 2014).

Questionnaire Measures

The BDI-II, a 21-item self-report inventory, was used to measure the severity of depressive symptoms (Beck, Steer, Ball, & Ranieri, 1996). In addition, before and after the experiment anxiety was assessed using the state version of the STAI (Van der Ploeg, 1982). Mood state was further measured using six Visual Analogue Scales (VAS) measuring how tired, energetic, angry, tension, depressed, and cheerful participants were feeling “at this moment.” The VAS is a 10-cm line, with endpoints from “not at all” to “very much.” Total mood scores were measured for each time point, where all six VASs were compounded in one score (from 0 to 10 in mm). The logic for pulling together all of our VAS scores in one compound measure is based on the Total Mood Disturbance Score index (TMDS; McNair, Lorr, & Droppelman, 1992), which is calculated by compounding the scales of the Profile of Mood States (POMS), including the fatigue and the vigor subscales (Vigor reverse-scored). We have explicitly chosen this strategy because in the literature it has been shown that this specific compound measure (TMDS) is highly correlated with State anxiety, capturing its fluctuations over time even better than the simple “tension” score (Bolmont & Abraini, 2001). Interestingly, this correlation with state anxiety is even higher during stress.

Critical Feedback Task

The critical feedback task (CFT) is a mental counting task where participants receive bogus negative feedback on their performance at the completion of each test-block. This paradigm was successfully used before with the aim of inducing state anxiety and distress (for details, see Rossi & Pourtois, 2012a, 2013, 2014; Baeken, Vanderhasselt et al., 2014). The on-screen instructions told the participants that this task measured perceptual learning abilities and sustained attention, reflecting general intelligence. The task was divided into a practice block and three test blocks, in which participants were asked to covertly count the number of deviant lines in a stream of standard lines, reporting this number at the end of each block. The standard lines were always tilted 35°, while the target lines had a different in-plane orientation. The angular difference between standards and targets was manipulated in order to create variation in perceptual load: one block was difficult (standard-target difference = 5° of angle), one was intermediate (standard-target difference = 10°), and one was easy (standard-target difference = 10°). Participants always started with the difficult block (unknown to them) and were informed by a cover story that after each block they would receive feedback on their performance, comparing them to a group of peers. Moreover, they were led to think that the difficulty of the subsequent block would depend on their performance on the current one (in a staircase design). However, the given feedback was in fact unrelated to performance (it was always negative), and the following block was always easier, to maintain motivation despite the elicitation of failure feelings and stress. Every feedback consisted of a neutral face with a text balloon, stating that they performed below average as compared to the other participants. A pseudorandomly generated scatterplot showed their own performance against the

1 For our study, we chose to only include women because of the homogeneity of our data, including men would require doubling the sample size to seriously consider gender as a factor in our design.

2 The logic for pulling together all of our VAS scores in one compound measure is based on the TMDS, which is calculated by compounding the subscales including the tired and the vigor subscales (it is important to know that our VAS subscales are based on the POMS subscales). We have explicitly chosen this strategy because in the literature it has been shown that this specific compound measure (TMDS) is highly correlated with state anxiety, capturing its fluctuations over time even better than the simple “tension” score (Bolmont & Abraini, 2001). Interestingly, the correlation with state anxiety is even higher during stress. Therefore, being particularly interested in the concepts of stress and state anxiety, we preferred to stick to a validated procedure (i.e., calculating the TMDS including fatigue and vigor), instead of creating a new compound score that had not been validated before in the literature. As a side note, given that anxiety has been related to processing efficiency impairments (see Derakshan & Eysenck, 2009), maintaining performance in the face of negative evaluations requires additional mental effort, which in turn can be expected to increase the perception of fatigue.
scores of the previous (alleged) participants. Since the targets were difficult to notice during the task, this brought a high uncertainty on one’s own performance, making it very likely for participants to believe that their performance was evaluated negatively. Moreover, the use of a direct comparison with other participants has been shown to be mostly effective in inducing stress/anxiety (Nummenmaa & Niemi, 2004).

Cardiac Activity: Heart Rate Variability

Heart rate was measured per beat with a telemetric heart rate monitor (Polar S810). The heart rate data were transmitted to a personal computer. Measurement errors were filtered with the Polar Precision Performance Software for Windows. The filter was set at a moderate filter power and a minimum protection zone of 6 beats per minute (Cottyn, De Clercq, Pannier, Crombez, & Lenoir, 2006). The resolution of a POLAR Vantage NV heart rate monitor, which is analogous to the S810 but with lower memory capacity, was studied in Kinnunen and Heikkilä (1998). The data were further analyzed with software (Kubios; Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) specifically designed for advanced HRV analysis. Artifacts were filtered on a medium level with the Kubios software (Tarvainen, Niskanen, Lipponen, Ranta-Aho, & Karjalainen, 2014). HRV can be described either by frequency or time domain indices. We used RMSSD (the root mean square successive difference of normal-to-normal intervals, in ms) as an index of HRV. RMSSD primarily reflects parasympathetically mediated short-term changes in heart rate and is one of the time domain indices recommended by a task force report on HRV measurement (The Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996) and has demonstrated to decrease as an effect of stress as well as worry (Delaney & Brodie, 2000; Pieper et al., 2007) and to increase as an indicator of successful emotion regulation (Thayer, Ahs, Fredrikson, Sollers, & Wager, 2012). For a detailed accounting of the other frequency bands see Thayer and colleagues (2010) for a recent review. HRV measurement started from the sixth minute after the start of the CFT, because the task only becomes stressful after the first negative feedback. The heart rate was recorded from the moment they entered the experimenter room, just before baseline, and stopped after the 15 min relax period at the end of the procedure.

HF-rTMS

A randomized sham-controlled, single blind, crossover design was used. To avoid carry-over effects from the previous stimulation, the second session was carried out after an interval of 3 days. The procedure of the second experiment day was exactly the same with the exception of the HF-rTMS session (real or sham), which was counterbalanced with random selection of order. HF-rTMS of the left and right DLPFC were performed using a MAGSTIM high-speed stimulator (Magstim Co., Whitland, UK) with a figure-of-eight shaped coil. To correct for individual anatomical differences and to avoid stimulation of other cortical areas besides the left or right DLPFC, all participants underwent a T1-weighted MRI (3D-TFE, voxel size 1 × 1 × 1 mm) of the brain using a 3-T Intera MR scanner (Philips, Best, The Netherlands). We located the left and right DLPFC visually in the 3D surface rendering of the brain based on the participant’s own gyral morphology and we marked the center of the middle frontal gyrus as the target site, which is anatomically localized in the center of the DLPFC (Brodmann area 9/46; Talairach coordinates—50, 34, 34). The corresponding coil position was found by determining the perpendicular projection of this point on the scalp (Peleman et al., 2010). We used the following stimulation parameters: 110% of motor threshold of the right abductor pollicis brevis muscle (stimulation intensity), 20 Hz (stimulation frequency), 40 trains of 1.9 s duration, separated by an intertrain interval of 12.1 s, resulting in 1,560 pulses per session. The total stimulation time was approximately 10 min. The study was conducted according to double-blind within-subjects design by randomized/counterbalanced crossover sham (placebo) and real HF-rTMS. Real and sham stimulation were performed at the same place on the skull, but for sham the figure-of-eight shaped coil was held at an angle of 90° only resting on the scalp with one edge. During stimulation, all participants wore earplugs and were blindfolded to ensure blindness of the stimulation procedure. The real and sham stimulation occurred immediately prior to the stress induction.

Procedure

Upon arrival on their first day the participants underwent an anatomical 3D MRI scan to define the exact stimulation point, which is based on a 10-min scan. At the start of the experiment, the polar equipment was put on and the participants were subsequently asked to fill in the STAI-State. Then, they were asked to relax for 20 min. After the 15 min (which allowed for 5 min of adaptation) HRV recorded baseline (T1), participants filled in the first series of VASs. Next, each participant received a single (sham or real) stimulation session in another room. When they returned they filled in their second VASs. Next, they were asked to perform the CFT, which took approximately 8 min (T2). After the task, they were given the third series of VASs. Next, the participants were asked to relax again for 15 min (T3). The HRV registration was stopped and participants received their final (fourth) VASs, together with the second STAI-State and the BDI. The procedure of the second day was similar to the first, except for the rTMS session (depending on which type of stimulation they received on the first day, e.g., Day 1 = Sham, Day 2 = Real; or Day 1 = Real, Day 2 = Sham). During the experimental procedure all participants were in a seated position, as well as for the real and sham stimulation sessions. After the experiment, all participants were fully debriefed and asked if they were aware of the purpose of the CFT. Some of the participants reported to be somewhat aware of the unsolvable nature of the task, but all said that they were stressed by the negative feedback they received. Furthermore, no significant differences were found when participants were asked to distinguish between real or sham HF-rTMS.

Statistical Analysis

All collected data were analyzed with SPSS 19 (Statistical Package for the Social Sciences). First, for mood analysis we used a 2 (experimental Condition: rTMS and sham) × 4(Time: after T1, before T2, after T2, and after T3) mixed analysis of variance (ANOVA) with stimulation side (left vs. right) as between-subjects
factor, followed-up by paired-samples t tests. To yield a sensitive measure of global impact of the protocol and so the CFT on negative mood, as proposed by Rossi and Pouthois (2012a, 2012b), we collapsed the VAS scores into a compound VAS for mood, by adding up the scores of the six items (the two positive mood items were reverse-scored): the compound score ranges therefore from a minimum of 0 (minimum level of negative mood) to a maximum of 60 (maximum level of negative mood; see Table 1 for an overview of the separate VAS scales). Our HRV data were not normally distributed (Shapiro Wilk test). To address this, we log transformed the data, which resulted in a normally distributed dataset, with all p values higher than .11. For the HRV data we performed a mixed 2 Condition (real vs. sham HF-rTMS) × 3 Time (T1 = baseline resting state HRV; T2 = stress induction HRV, and T3 = recovery resting state HRV) × 2 Stimulation Side (Left vs. Right) ANOVA, with time and condition as within-subjects factors, stimulation side as between-subjects factor, and HRV as dependent variable. To follow-up interaction effects, paired sample t tests were performed. Effect sizes were calculated as Cohen d (Cohen, 1988) for the HRV means (based on the observed means and standard deviations), for both within as between-condition comparisons.

Results

Mood

The ANOVA yielded a marginally significant effect of time, \( F(3, 35) = 2.54; p = .060 \). Given the absence of simulation side and condition interaction effects, as a follow-up we computed the means collapsed over condition and stimulation side of the total mood scores, to follow up the near significant main effect of time. As shown in Figure 1, negative mood increased significantly between T1 and T4, \( t(37) = 2.05; p = .048 \), with a marginally significant increase between T2 and T3 \( t(37) = 1.91; p = .064 \), that is, before and after the CFT. This shows a global increase of negative mood throughout the whole procedure, while the increase between T2 and T3 shows an increase of negative mood, specifically during the CFT. Next, we also compared the possible mood effects on the STAI-State questionnaire before and after the protocol. In the left stimulation study one participant failed to complete the STAI questionnaire. The ANOVA with the STAI-State score as dependent variable showed no significant main or interaction effects (all \( p’s > .09 \)).

HRV

The \( 2 \times 3 \times 2 \) ANOVA showed a main effect of time, \( F(1, 35) = 11.59; p < .001 \) and an interaction effect of time and condition, \( F(1, 35) = 4.04; p = .026 \). The crucial three-way interaction effect between condition, time and stimulation side, \( F(1, 35) = 4.63; p = .016 \) was also significant. To further follow-up this interaction effect, we performed separate \( 2 \times 2 \) within-subjects ANOVAs for the left and the right side stimulation group.

For the left side stimulation group, no significant main effects for both condition, \( F(1, 18) = 1.20; p = .29 \), and Time, \( F(1, 17) = 2.16; p = .15 \) emerged. Most importantly, a highly significant interaction between condition and time was observed, \( F(1, 17) = 8.75; p = .002 \). To follow-up this interaction effect, paired sample t tests were performed. At T1 there was no significant difference in RMSSD between the real HF-rTMS and the sham condition, \( t(18) = .85; p = .41 \). However, at T2 (during the stress task) we observed a significant higher RMSSD in the HF-rTMS-condition as compared to the sham condition, \( t(18) = 3.26; p = .004 \). Finally, at T3 the significant difference in RMSSD between HF-rTMS-condition and sham-condition disappeared, \( t(18) = .69; p = .50 \). When looking at the within condition differences, we found a significant increase in RMSSD in the rTMS condition from T1 to T2, \( t(18) = 3.05; p = .007 \) and from T2 to T3, \( t(18) = .28; p = .78 \). As such, a single rTMS session, successfully led to an increase of RMSSD during the stress induction. In the sham condition there was a marginally significant decrease in RMSSD from T1 to T2, \( t(18) = 1.97; p = .065 \) and from T2 to T3, \( t(18) = .18 \). Comparing T2 with T3, a significant increase in RMSSD was found in the sham

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<td>Visual Analogue Scales Descriptives (in cm)</td>
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<td><strong>Condition</strong></td>
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*Note.* Mean ratings and standard deviations for the Visual Analogue Scale (VAS) subscales before (T1) and immediately after high-frequency repetitive Transcranial Magnetic Stimulation (HF-rTMS)/sham (T4). Immediately after the critical feedback task (CFT) (T5) and 15 min later (T6). (See Figure 1 for graphical presentation of total mood scores). VAS scales are used to capture mood states, and scores are expressed on scales going from 0 to 10 (cm) and range from 0 (absence of that emotion) to 10 (max emotion).
sessions had also no significant effect on the stress response, the order of presentation of rTMS versus sham conditions across sessions had no significant effect on the stress response, $F(1, 16) = .25; p = .78$.

For the right side stimulation group, no significant interaction between condition and time was observed, $F(1, 17) = .99; p = .42$. The order of presentation of rTMS versus sham conditions across sessions had also no significant effect on the stress response, $F(1, 16) = .48; p = .63$. The pattern of results is shown in Figure 2.3

### Discussion

The present study was designed to investigate the effects of a single HF-rTMS session over the left and right DLPFC on the physiological stress response of healthy female participants. The results showed the expected significant increase of HRV during stress induction compared to baseline after left-sided stimulation, indicative of the mood inducing effect of the CFT over condition and stimulation side.

During the recovery period after the whole left side stimulation procedure, the differences between sham and real stimulation disappeared, which means that our healthy participants showed normal stress recovery after the stressor has disappeared. In addition, although the mood analyses did not show a three-way interaction, the time effect shows a general impact of the protocol, with an increase of the total negative mood score from before to after the stress induction task, indicative of the mood inducing effect of the CFT over condition and stimulation side.

The present study was the first experimental study using HF-rTMS over the left DLPFC demonstrating an impact on parasympathetic modulation in humans. However, through which exact pathway left DLPFC HF-rTMS affects the ANS remains to be clarified and without concomitant neuroimaging techniques the interpretation of our psychophysiological results remains to some extent speculative. A possible working mechanism points to a DLPFC/anterior cingulate cortical (ACC) pathway. Indeed, in brain imaging studies examining negative affect, besides the dorsolateral prefrontal cortex, dorsal (d)ACC areas are often involved as well (Pizzagalli, 2011). Diminished connectivity between the DLPFC and dACC might result in the failing of the ACC’s inhibitory role in amygdala regulation of emotional processing in major depression. Different brain imaging studies in MDD lend support to the assumption that left HF-rTMS affects and normalizes DLPFC and ACC metabolic and functional neuronal activities (Baeken et al., 2009; Kito, Hasegawa, & Koga, 2012; Fox, Halko, Eldaief, & Pascual-Leone, 2012). The subgenual (sg)ACC, part of the ventromedial prefrontal cortex (Barbas et al., 2003; Ray & Zald, 2012), may be a critical region to be involved in the response to HF-rTMS. In addition, in Baeken et al. (2015) PET imaging was used to show the impact of HF-rTMS on the subgenual ACC in refractory unipolar major depression, whereas in Baeken, Marinazzo et al. (2014) the effect of rTMS on functional connectivity between prefrontal areas and the subgenual ACC was demonstrated. The sgACC, implicated in “visceromotor” functions and in modulating affect, has consistently been shown to be metabolically hyperactive during depressive episodes and successful antidepressant treatment results in neuronal attenuation of this ventromedial prefrontal cortical area (Drevets, Savitz, & Trimble, 2008). Furthermore, HF-rTMS treatment has been shown to affect deregu-

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3 Given that RMSSD, PNN50, and the high frequency component of the power spectrum (HF power) are closely related, and all reflect vagal cardiac influence, we included the HF-HRV (frequency domain methods) and PNN50 (time domain methods) in a similar mixed 2 × 3 × 2 ANOVA. The results show converging findings to the RMSSD data. Furthermore, all indices of HF-HRV, PNN50, and RMSSD correlated highly (all $r > .800$; $p < .001$).
lateral sgACC neurocircuits in depressed patients (Baeken, Vanderhasselt et al., 2014, 2015; Fox, Buckner, White, Greicius, & Pasqual-Leone, 2012). Given that cortical brain systems that are hypothesized to regulate cardiac autonomic activity during behavior include the medial-prefrontal regions of the cortex (Gianaros et al., 2004), our HRV findings are in line with the existing animal literature showing an attenuating effect of rTMS of frontal brain regions (e.g., the medial-prefrontal cortex). HRV findings are indicative of the potential of rTMS to increase cognitive control over stress responsiveness. Moreover, these findings are consistent with the conclusions of Davidson et al. (2002) and Maier et al. (2006) that the PFC is implicated in affect regulation and is vital for coping with stress inducing events. Indeed, the increase in HRV associated with emotion regulation has been related to cerebral blood flow changes in the PFC (Lane et al., 2009). Our results are consistent with the neurobiological correlates of vulnerability for depression that an important therapeutic aim would be to restore stress reactivity. These results have general clinical relevance because it has been recorded that a greater reduction in cardiac parasympathetic activity during psychological stress, as reflected by a greater reduction in (vagal mediated) HRV, may be a potential risk factor for subclinical atherosclerosis, particularly in the coronary arteries (Gianaros et al., 2005). HR reactivity and cardiovascular reactivity also predict other health-related effects of stressors on the body (Wager et al., 2009). However, further research using rTMS combined with the presentation of a stressor to probe the physiological stress system in MDD and other disorders is necessary. In line with the beneficial use of exposure in the treatment of various disorders, and the crucial role of the PFC in these effects (for a review, see De Raedt, 2006), it might well be that the effects of rTMS can be boosted by combining it with exposure to a stressor. Furthermore, we believe that these results show the potential of rTMS to be an additional treatment protocol, next to the more conventional (therapeutic and medicinal) treatments of depression.

An important caveat is that the absence of a physiological stress response in the right side stimulation group during the sham session, which is difficult to explain. One possible reason might be that there was no randomization for group (first left stimulation group, then right). Given that high vagally mediated HRV is associated with cognitive, emotional, and autonomic self-regulatory capacity (Thayer et al., 2000, 2009), and people with high HRV cope better with stress, this might explain the absence of a decrease of HRV (and increase in HR) for the right stimulation group. Because the overall HRV was larger in the right stimulation group.
as compared to the left stimulation group, a ceiling effect might prevent the stress induction task to influence their physiological responses. However, we cannot formally exclude that this group, by coincidence, coped better with the induced stress, explaining the absence of the physiological stress response in the sham condition in the right stimulation group, compared to the left. That said, future research should focus on this randomization issue when conducting lateralization studies.

Some limitations should be noted when interpreting our results. First, in the present study only young (18–30 years) healthy women were tested. Although this had the advantage of selecting participants known for their higher emotion sensitivity, it limits generalizability. In addition, there is also evidence that men and women differ in their autonomic and cardiovascular responses to psychological stressors (Matthews & Stoney, 1988). Future work is thus needed to test whether the present results generalize to a male sample or to a sample with a larger variation in age and health (e.g., MDD). Second, the fact that HF-rTMS did not induce a subjective differential effect (sham vs. real stimulation) on the mood scales might be considered as surprising. However, subjectively experienced mood gives only limited insight into the neurophysiology of emotion and physiological responses might operate independently of verbal reports (Buck, 1999; Campbell & Ehler, 2012). Thus, this may be indicative of a difference between subjective experience of distress and physiological responses. Furthermore, the menstrual cycle phase could be another systematic interindividual influence, which may have impact on HRV (e.g., Sato, Miyake, Akatsu, & Kumashiro, 1995) but was not controlled in the present study. We suggest that this issue should be addressed in future research. Moreover, other factors, such as respiratory rate, tidal volume, or momentary physical activity may influence a reliable estimation of cardiac vagal tone (for an extensive discussion, see Grossman & Taylor, 2007). However, given the consistency between our different measures (RMSSD, HF-HRV, PNN50), these factors seem to have played a minor role. Third, all analyses are based on RR interval data, no ECG signal data or respiratory data was collected. Given the influence of respiratory changes on HRV (Cyranowski, Hofkens, Swartz, Salomon, & Gianaros, 2011), these results should be replicated with proper respiratory (and ECG) data included, or this should be taken into account when interpreting and generalizing the findings.

To summarize, the results of this study suggest that the DLPFC plays a significant role in the modulation of stress responses in healthy participants. In addition, this study also shows that through the use of HF-rTMS over the left DLPFC we can possibly augment the cognitive control to cope with emotional stimuli, which is highly relevant in the treatment of stress-related disorders (Scher et al., 2005).

References


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