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Theta-burst stimulation and frontotemporal regulation of cardiovascular autonomic outputs: The role of state anxiety

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ABSTRACT

Dysregulation of autonomic cardiovascular homeostasis is an important cardiological and neurological risk factor. Cortical regions including the prefrontal and insular cortices exert tonic control over cardiovascular autonomic functions. Transcranial Magnetic Stimulation (TMS) may be a suitable approach for studying top-down control of visceromotor processes. However, there is inconsistent evidence as to whether TMS can modify cardiovascular autonomic states. One reason for the inconsistency may arise from the lack of studies accounting for the acute affective states of participants with respect to the stimulation procedures. To gain more insights into these processes, we evaluated the effects of intermittent and continuous theta-burst stimulation (TBS) to the right frontotemporal cortex on state anxiety and cardiovascular responses in a preliminary study. State anxiety significantly increased for both intermittent and continuous TBS relative to sham. Intermittent TBS also significantly increased heart-rate variability (HRV) at natural and slow-paced breathing rates. The effect of intermittent TBS on vagally-mediated HRV was attenuated after accounting for stimulation-induced anxiety, suggesting that increased HRV after stimulation may reflect a response to a transient stressor (i.e., the stimulation itself), rather than TBS effects on visceromotor networks. In contrast, continuous TBS increased pulse transit time latency across breathing rates, an effect that was enhanced after accounting for state anxiety. TMS is a promising approach to study cortical involvement in cardiovascular autonomic regulation. The findings show that TBS induces effects on visceromotor networks, and that analysis of state covariates such as anxiety can be important for increasing the precision of these estimates. Future non-invasive brain stimulation studies of top-down neurocardiac regulation should account for the potential influence of non-specific arousal or anxiety responses to stimulation.

1. Introduction

Effective regulation of blood volume, arterial pressure, and heart rhythm is critical for maintaining cardiovascular homeostasis. Impaired cardiovascular regulation may lead to conditions such as hypertension, which is the leading risk factor for global disease burdens (Bromfield and Muntner, 2013). Many lines of evidence point to neural mechanisms in the pathogenesis of hypertension, arrhythmias and heart failure (Mancia and Grassi, 2014; Shen and Zipes, 2014). For instance, blood pressure and cardiac rhythm are regulated via sympathetic and parasympathetic pathways, which are under the control of brainstem and midbrain reflexes involving the hypothalamus, periaqueductal gray (PAG), nucleus of the solitary tract (NST), vagal and sympathetic motor nuclei, among other regions (Silvani et al., 2016). However, the cortex also exerts an influence on tonic and phasic autonomic outflows. In particular, activity of the medial and orbital prefrontal cortices, the ventrolateral prefrontal, insular and opercular cortices have been consistently associated with heart rate and heart rate variability (Vargas et al., 2016; Thayer et al., 2012), baroreflex loading and unloading (Kimmerly et al., 2006; Goswami et al., 2012), and muscle sympathetic nerve firing (Macefield and Henderson, 2016). Lesion studies further highlight the role of the insula in cardiovascular control- acute ischemic stroke affecting the insula is associated with severe cardiac arrhythmias (Seifert et al., 2015), depressed heart rate variability (Colivicchi et al.,

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2004), and baroreflex impairment unexplained by atherosclerosis (Sykora et al., 2009). Thus, the insula and prefrontal cortices are key cortical regions of a cardiovascular viscerosensory and visceromotor network.

Noninvasive brain stimulation (NIBS) may be useful to enhance understanding of cortical involvement in cardiovascular autonomic regulation in relation to illness and health. Previous physiological and psychophysiological studies have indicated that transcranial magnetic stimulation (TMS) of cortical regions may modulate cardiovascular autonomic responses. For instance, Macefield and colleagues (Macefield et al., 1998) reported inhibition of muscle sympathetic nerve activity after cardiac synchronous single-pulse TMS applied to the vertex or motor cortex, while Berger et al. (2017) reported enhanced cardiac deceleration in response to affective pictures after repetitive TMS (rTMS) to the right dorsolateral prefrontal cortex (DLPFC). The potential for TMS to modulate the cortical-autonomic network may also be relevant to the treatment of hypertension (Cogiamanian et al., 2010) and stress-related psychiatric disorders which are associated with depressed cardiac vagal function and increased risk of cardiovascular disease (Gianaros and Sheu, 2009; Ginty et al., 2017; Thayer and Lane, 2007). However, there is currently only very limited prospective evidence that rTMS treatments may improve cardiac vagal function in clinical populations (Udupa et al., 2007; Udupa et al., 2011).

Although several studies indicate positive effects of (r)TMS on cardiovascular autonomic responses in both clinical and healthy populations, the findings remain equivocal: the recent publication of two meta-analyses (Schestatsky et al., 2013; Makovac et al., 2017) which amalgamated a highly heterogeneous body of literature, arrived at somewhat divergent conclusions regarding the effects of NIBS on autonomic cardiovascular control. Schestatsky et al. (2013) could not identify consistent effects of TMS and related parameters on autonomic responses in general, although there was some evidence that HRV is most sensitive to TMS effects on the autonomic nervous system relative to other autonomic response systems, although the magnitude of this effect was not clear. Makovac et al. (2017) instead identified a moderate effect size for the influence of TMS on heart rate reductions and increases in high frequency heart rate variability (HF-HRV), and a small effect size for blood pressure reductions. In addition, Makovac and colleagues identified the prefrontal cortex as the relevant area for stimulation compared to other sites, (such as the motor cortex), although studies targeting different brain regions are lacking. There are differences between these analyses that may have accounted for the divergent conclusions. The Schestatsky et al.'s study was semi-qualitative (i.e. in terms of the “frameworks” for partitioning groups of studies for meta-analytic assessment), and focused on which brain stimulation parameters may best induce autonomic responses, whereas the Makovac and colleagues used a more sensitive and rigorous quantitative approach that exclusively focused on cardiovascular autonomic response effect sizes over methodological variability across studies. Furthermore, these meta-analyses are distinct in that Makovac et al. included only NIBS studies that measured heart rate, heart rate variability, and blood pressure, whereas Schestatsky et al. included studies measuring any autonomic response system, including skin conductance, cortisol, body temperature, respiration, etc., resulting in greater heterogeneity of studies included in their analysis.

Despite the divergence, both meta-analyses identified several pervasive methodological limitations within the research they reviewed, including: an insufficient number of placebo-controlled studies, underutilization of autonomic perturbation tasks (e.g. tilt-table test, Valsalva maneuver, etc.), lack of diversity in sites of stimulation, limited timescales on which responses were measured (typically concomitant to the stimulation), and importantly, failure to assess whether acute stress or arousal induced by the neurostimulation procedures were sufficient to drive any observed effects. This last point warrants careful attention since the sensations of verum stimulation can be arousing or stress-provoking, which may alter autonomic responses in parallel during experimental measurements, or worse, entirely account for observed effects. The potential impact of such covariates raises the possibility that the effects identified in Makovac and colleagues' meta-analysis could be influenced by such confounds. Ideally, sham stimulation controls for the sensory experience associated with (r)TMS procedures (which have the potential to induce anxiety or arousal), however the sham coils and other placebos currently used are often not adequately matched in this respect (Duecker and Sack, 2015). Although a single-session of rTMS is usually not found to acutely affect mood in healthy volunteers (Remue et al., 2016) it has been reported that state anxiety prior to stimulation (perhaps related to expectations concerning the TMS procedures) affects both cognitive-affective and cortisol responses to rTMS (Baeken et al., 2011; Vanderhasselt et al., 2011). These findings emphasize the need to examine the influence of state anxiety as a covariate in non-invasive brain stimulation studies of cardiovascular autonomic regulation.

The present study examined possible differential effects between continuous and intermittent theta burst stimulation (cTBS and iTBS) to a right frontotemporal target on HRV and pulse transit time [PTT] in a sham-controlled, repeated measures design. Similar to rTMS, theta-burst stimulation (TBS) protocols may induce long-term potentiation or long-term depression-like effects, but may also produce more enduring effects on cortical excitability (Lizbeth et al., 2010). Consequently, theta-burst protocols may be more effective than rTMS at achieving tonic changes in autonomic balance under resting conditions. iTBS protocols have, to our knowledge, not yet been used to examine its effect on neural autonomic cardiovascular control. However, cTBS has been used to examine neural cardiac interoception. Pollatos et al. (2016) reported reductions in heart-beat detection accuracy and altered amplitude of the heart-evoked potential (HEP) in frontocentral electrode sites after cTBS of a right frontotemporal target. The HEP is an endogenous evoked-potential that reflects neural processing of cardiac afferents (Gray et al., 2007), which intracranial EEG studies have localized to the insula and operculum (Park et al., 2017). Therefore, cTBS of this right-lateralized frontotemporal target appears capable of modulating neural cardiovascular processing. However, Pollatos and colleagues did not report whether cTBS affected autonomic outflows, nor could they determine whether iTBS may exert distinct, or even similar effects compared to cTBS.

As there is no a priori basis for assigning directional effects on cardiovascular responses to the right frontotemporal cortex depending on TBS frequency, our hypotheses for this preliminary study were non-directional. We expected to find an enhancement of HRV for at least one of the TBS stimulation protocols after stimulation to the right frontotemporal cortex. In addition to HRV, we also examined PTT, a surrogate beat-to-beat measure of systolic blood pressure (albeit a noisy measure that is also influenced by the cardiac pre-ejection period (PEP)) (Payne, 2006). We expected an increase in PTT latency in response to TBS for at least one of the TBS stimulation protocols, which may reflect a reduction in systolic blood pressure or greater PEP duration. HRV and PTT were further assessed under two breathing conditions consisting of slow-paced breathing and spontaneous breathing. Slow paced-breathing at 0.1 Hz is an autonomic challenge which is believed to generate large-amplitude resonance power in the baroreflex feedback loops (Lehrer et al., 2000). It is reflected as a large increase in respiratory sinus arrhythmia (RSA) spectral power centered at 0.1 Hz. In healthy individuals, RSA power during 0.1 Hz breathing has a correlation of 0.77 with baroreceptor sensitivity (BRS) measured using the Finapres method (Davies et al., 2002). Thus, RSA power during 0.1 Hz breathing can also be taken as a proxy measure of baroreceptor gain on heart rate, which is expected to increase with stimulation. Finally, we evaluated the influence of stimulation-provoked state anxiety. We expected that state anxiety will increase in response to verum stimulation relative to sham, and that state anxiety will at least partially account for effects of TBS on cardiovascular state for any given cardiovascular index and breathing condition.

2. Methods

2.1. Participants

Twenty-eight participants were recruited through a Ghent University social media platform dedicated to advertisement of psychology and neuroscience studies. Four were excluded from the study due to the following reasons: failure to meet inclusion criteria during the Mini International Neuropsychiatric Interview (MINI) (n = 1) (Sheehan et al., 1998), frequent ectopic beats observed during visual inspection of the electrocardiogram (ECG) trace (n = 1), or lack of tolerance to the physical sensations of TBS protocols (n = 1) or motor threshold (MT) stimulation (n = 1). The remaining participants included 24 individuals (14 female, 10 male; ages: M = 25.39, SD = 6.15). All retained participants were right-handed, physically healthy and non-smoking. A few participants reported having previously participated in TMS experiments, however the large majority were naïve to TMS. Participants were free of contraindications for TMS (including personal or family history of epilepsy, migraine, implanted medical devices, pregnancy), and free of other medical conditions including cardiovascular, pulmonary, metabolic, neurological and psychiatric disorders, and not experiencing any current serious psychosocial distress, such as recent death of a family member, as determined by a pre-screening health questionnaire and MINI examination. No subject was using prescribed or over-the-counter medications, apart from hormonal birth control pills in women. Written informed consent was obtained and study procedures were approved by the University of Ghent Ethics Committee. Participants were financially compensated.

2.2. Study protocol

Potential participants were first screened via email for TMS contraindication and other exclusion criteria. If eligible, they were scheduled for three visits that were spaced at least four days, but less than ten days apart. All testing sessions took place in the afternoon to minimize potential circadian influences. Participants were asked to refrain from alcohol and strenuous exercise for at least 24 h prior, to avoid caffeine at least 4 h prior, and to wear comfortable clothing. After consent, the MINI Interview was given to further rule out the presence of any mental health history. Participants were then familiarized with the six-minute Slow Breathing task, in which the rate of inspiration and expiration was guided by an animated geometric object presented on a computer screen. The object expanded during the period of inspiration (5.0 s) and shrunk during the period of expiration (5.0 s), resulting in a complete cycle (and breathing rate) of 0.1 Hz. We verified that each participant could engage the diaphragm during slow breathing, match the oscillations of the object at the appropriate phase, and breathe comfortably and naturally, without hyperventilation or light-headedness. The other condition consisted of 6 min of Spontaneous Breathing, during which the participant was instructed to direct their gaze to a static black fixation cross on the center of a gray screen and not attempt to actively manipulate their breathing pattern. To facilitate spontaneity, subjects were advised that they could let their mind wander during this period.

Electrocardiograms (ECG), pneumogram (RESP), finger pulse photoplethysmogram (PPG) were acquired with a 1000 Hz sampling rate with the Biopac MP150 system and Acqknowledge software. The animation and fixation cross for the Slow and Spontaneous Breathing conditions, respectively, were presented via computer screen using Psychtoolbox 3.0 and MATLAB (The MathWorks Inc., Nantucket, MA). Three electrodes were attached for measurement of Lead II ECG. RESP was measured using a strain-gauge transducer placed around the abdomen. PPG was measured by attaching the transducer on the middle finger of the left hand.

Subjects were seated in a reclining chair with legs and feet raised to approximately the same level as the hips, with their hands resting either at their side or on their lap. Participants practiced slow breathing prior to stimulation. The goal of the practice was to provide enough time for each subject to achieve hemodynamic stability and to enter into a proper mental state for the task. This was intended to reduce sources of variability of subjects' physiological and mental states upon arrival to the lab. After this period, we prepared the subject for stimulation, which is described in detail in Sections 2.3 and 2.4 below. Immediately after theta-burst or sham theta-burst stimulation, subjects reported their state anxiety (STAI_TBS) using the State subscale of the commonly used State-Trait Anxiety Inventory Y (STAI-Y) (Spielberger, 1983). STAI-Y State consists of 20 questions that evaluate the respondent's current state of anxiety by asking “how do you feel right now” using items that measure subjective experiences of nervousness, worry, apprehension, autonomic arousal, fear, and tension on a 4-point Likert-type scale. Higher scores indicate greater state anxiety. STAI-Y State was chosen as the means of estimating state anxiety based on previous literature from our group indicating that baseline state anxiety using this measure affects hypothalamic-pituitary-adrenal axis and attentional bias responses to rTMS applied to prefrontal targets (Baeken et al., 2011; Vanderhasselt et al., 2011). After reporting state anxiety, participants then performed the breathing task: first Slow, then Spontaneous Breathing in a fixed order. Physiological recordings were taken during this period. After completing the breathing tasks, subjects again reported their state anxiety (STAI_POST), at which point the session was concluded. At the end of the third testing day, participants were debriefed.

2.3. Motor threshold testing and stimulation site

Motor threshold testing occurred only on the first testing day. To establish individual motor thresholds we used the visual method of limits to identify the minimum intensity required to produce a visible twitch in the abductor pollicis brevis in 5/10 consecutive trials (Pridmore et al., 1998; Varnava et al., 2011).

The site of stimulation was determined using the international 10–20 EEG system heuristic introduced by Pollatos et al. (Pollatos et al., 2016) for targeting the right anterior insula (aINS) for a study of cardiac interoception (which we describe in the present report as a frontotemporal region). Specifically, the figure-of-eight coil was positioned over the right frontotemporal locations that built a triangle corresponding to the 10–20 positions F8, FC6, with the center-top of the coil pointing to F6 (with the handle of the coil pointing towards FT10). See Fig. 2 for an illustration of the electrode sites, and see Pollatos et al. (2016) for additional details. After fitting the EEG cap, we drew points on the subject's scalp with a marker under the electrode positions indicating the points along which to orient the coil. The cap was removed before stimulation.

2.4. Theta-burst stimulation parameters and hardware

Continuous, intermittent, or sham TBS was delivered in randomized order for each subject at 100% MT. The stimulation was applied using a Magstim Rapid^2 Plus^1 magnetic stimulator (Magstim Company Limited, Wales, UK) with an active and a sham 70 mm Double Air Film figure-of eight-shaped cooled coil. The Magstim 70 mm Double Air Film sham coil is identical to its active variant in all but stimulation output. Each session delivered 600 pulses consisting of a burst of three stimuli at 50 Hz, repeated every 200 ms. Continuous theta burst stimulation (cTBS) consisted of 600 consecutive pulses applied in 40 s, while for intermittent theta burst stimulation (iTBS), the 600 pulses were delivered in separate trains with a duration of 2 s and an inter-train interval of 6 s for a total of 160 s in accordance with (Huang et al., 2005). The stimulation parameters for sham were randomly assigned to follow either the continuous or intermittent theta burst pattern for each individual (12 subjects received sham-iTBS, 12 subjects received sham-cTBS). See Fig. 2 for an overview of the study design.

2.5. Physiological measurement

2.5.1. Heart rate variability

The data processing was performed offline using in-house custom scripts with MATLAB. The peaks of the ECG R-wave were identified separately for Spontaneous and Slow Breathing conditions using in-house developed MATLAB scripts centered around the built-in functions *filtfilt* to detrend the ECG signal with a no phase distortion Butterworth filter and *findpeaks* to identify the peaks of the R-waves. Recordings were manually inspected for errors in R-peak identification and ectopic beats. No errors or ectopic beats were found. The inter-beat intervals were computed and HRV was determined in accordance with the HRV Task Force guidelines (Task Force, 1996) and the Kubios software user's guide (Tarvainen et al., 2014). Analysis of power spectral density was then carried out using the Fast-Fourier Transform with default settings in Kubios: high-frequency band 0.15–0.4 Hz; low-frequency band 0.04–0.15 Hz; and very low-frequency band 0.0–0.04 Hz. The estimates of spectral density for each frequency band (in milliseconds squared per Hz) were transformed logarithmically. Additionally, we extracted the temporal domain index Root Mean Square of Successive Differences (RMSSD).

In the spontaneous breathing condition, RSA power in the high frequency band reflects vagal influences on heart rate, assuming breathing rate is above 0.15 Hz (Task Force, 1996). During slow breathing, RSA shifts to the low-frequency band and no longer purely reflects vagal influences on heart rate, but instead reflects a mixture of vagal efference and resonance power in the baroreflex feedback loops (Davies et al., 2002; Vaschillo et al., 2006). The temporal domain index RMSSD acts as a high-pass filter on the interbeat interval time series and can be assumed to reflect parasympathetic effects on heart rate. RMSSD is less sensitive to respiratory rate compared to spectral-domain indices (Penttilä et al., 2001; Schipke et al., 1999). Thus, low-frequency HRV power (LF-HRV) was extracted for the Slow Breathing condition, whereas RMSSD was extracted for the Spontaneous Breathing condition.

2.5.2. Pulse transit time

Pulse transit time (PTT) refers to the time it takes for blood to travel between two arterial sites. To measure PTT, the R-wave of the ECG is used as a starting point, and the upslope of the PPG wave is used as the end point. However, there is a short delay between the occurrence of the R-wave and the opening of the aortic valve (PEP). Hence, PTT measured using the R-wave includes the PEP time interval which may account for 12%–35% of the PTT measurement (Payne, 2006). PEP is influenced by beta-adrenergic stimulation, which shortens PEP (although it is also influenced by blood pressure and ventricular stroke volume) (Smith et al., 1999). Nevertheless, PTT provides a useful dynamic, beat-to-beat estimate of cardiovascular processes that has a strong inverse correlation with systolic blood pressure, but a less reliable association with diastolic or mean arterial pressure (Payne, 2006; Wibmer et al., 2014; Gao et al., 2016). For our analyses, the ECG R-wave was used as the starting point for estimation of PTT. We used the peaks of the first derivative of the zero-phase shift Butterworth low-pass filtered PPG series to identify the point of arrival of the arterial pulse-wave, and then computed the time difference between the peak of the differentiated PPG wave and the corresponding R-wave following for all successive beats. The mean of the PTT series in milliseconds was used for subsequent statistical analyses.

3. Data analysis

3.1. Statistical analysis

Linear mixed effects regression (LMER) was used. All analyses were carried out using R Statistical Software v3.3.2 (R Development Core Team, 2016). LMER models were computed using the package '*lmerTest*' (Kuznetsova et al., 2017). For random effects, we included intercepts for each subject, and stimulation condition was entered as the fixed effect. Sham was set as the reference level for Stimulation (sham, iTBS, cTBS). Thus, cTBS and iTBS should be interpreted with respect to sham. Response variables were the autonomic responses after stimulation (LF-HRV during Slow Breathing, RMSSD during Spontaneous Breathing, or PTT for both conditions). To determine whether anxiety induced by stimulation accounts for the effects of stimulation on HRV and/or PTT, state anxiety immediately after stimulation (STAI_TBS) was entered as a fixed effect covariate in a subsequent set of regressions. To assess the significance of theta-burst stimulation and STAI_TBS, *lmerTest* provides *p*-values for the fixed effects using the Satterthwaite approximations to degrees of freedom. Confidence intervals for fixed effects were estimated with bootstrapping using the *confint* function. The contrasts for cTBS versus iTBS (i.e. the difference cTBS – iTBS) were obtained through the least squares means of the fitted models, also computed from the *lmerTest* library. Lastly, LMER was also used to assess whether any increase in state anxiety due to stimulation was transient and/or particular to the type of stimulation. To test this, we modeled the main fixed effect of Stimulation (cTBS, sham, iTBS) with sham as the reference level and Time (TBS, Post) with TBS as the reference level (where TBS refers to the period immediately after stimulation, and Post refers to the period of time after completing the breathing tasks), as well as a fixed-effect interaction between Stimulation and Time to test whether the change in state anxiety immediately following stimulation to the end of the breathing tasks was different for iTBS and cTBS relative to sham. Additional analyses were run to examine Pearson's correlations (2-tailed tests) among physiological responses and STAI_TBS scores. Slow and Spontaneous Breathing conditions were modeled separately.

3.2. Effect size estimation for mixed effects models

R^2 was computed for each model using Nakagawa and Schielzeth's (2013) method. The approach yields both the marginal and conditional effect sizes (that is, for the fixed effects and for the fixed plus random effects, respectively), and overcomes the problems associated with most definitions of marginal R^2 for mixed effects models, such as decreasing or negative values. The values were obtained using the R function *r.squared.GLMM* from the package MuMln which implements the method.

3.3. Missing data

There were three participants for whom two of the three experimental sessions were available. STAI_TBS was missing three data points. Instances where the PPG signal was of poor quality due to technical issues or corrupted with many movement artifacts were removed since it would not be possible to reliably compute PTT on those segments ($n = 2$ for Slow Breathing, and $n = 1$ for Spontaneous Breathing).

4. Results

4.1. State anxiety

There was a significant main effect of Stimulation on state anxiety. Specifically, both cTBS and iTBS increased state anxiety relative to sham (cTBS: $p = .026$; iTBS: $p = .003$). There was an additional main effect of Time, in which anxiety at the end of the breathing tasks was significantly lower than state anxiety immediately after stimulation ($p = .003$), suggesting that the anxiety promoting effect of stimulation was transient. As the interaction terms were not significant, there were no differential changes in anxiety from stimulation to the end of the breathing task that depended on whether the stimulation was continuous or intermittent (cTBS * Post: $p = .199$; iTBS * Post: $p = .117$). The fixed (marginal) effects of Stimulation, Time, and their interaction

Table 1
LMER fixed effects examining changes in state anxiety from immediately after Stimulation to completion of the breathing tasks (Post) for each experimental day.

State anxiety	FE	Lower-95% CI	Upper-95% CI	SE	*p*-Value
Intercept	31.78	28.7	34.8	1.51	< .00001
cTBS	3.53	0.60	6.73	1.56	**.026**
iTBS	4.51	1.52	7.9	1.5	**.003**
Post	−4.56	−7.96	−1.65	1.52	**.003**
cTBS ∗ Post	−2.79	−7.03	1.29	2.15	.199
iTBS ∗ Post	−3.31	−7.48	0.69	2.09	.117

explained 20.6% of the variance in state anxiety, while overall (conditional) model explained 60.9% of the variance in state anxiety. See Table 1 for an overview of the LMER fixed effects. Average state anxiety immediately after each stimulation condition was Sham: (M = 31.57; SD = 7.85), cTBS: (M = 35.62; SD = 8.66), iTBS: (M = 36.29; SD = 10.26), with a range of scores from 20 to 59. Fig. 3 illustrates the differential levels of state anxiety after each stimulation condition.

4.2. Slow breathing

4.2.1. LF-HRV

For the LMER model including only TBS, there was a significant main effect of iTBS (p = .03) such that iTBS increased HRV power in the LF range during slow breathing. However, cTBS did not significantly change LF-HRV (p = .52). STAI_TBS was not significant (p = .54), nevertheless, adding it as a covariate suppressed the significance of iTBS on LF-HRV (p = .06). The fixed effect of TBS explained only 2.4% of the variance in LF-HRV (conditional explained variance was 71.4%). Adding STAI_TBS to the model did not appreciably increase the explained variance in LF-HRV (marginal: 2.8% and conditional: 71.5%). The least squares mean differences between cTBS and iTBS were not significant in either model. See Table 2 for LMER estimates.

4.2.2. PTT

For the model including only TBS, cTBS was a significant predictor of PTT (p = .05) indicating an average increase in PTT latency by 13.7 milliseconds. iTBS did not exert a significant effect on PTT (p = .93). With STAI_TBS added, cTBS remained significant and the effect was enhanced (p = .029), with the average latency increased to 16.5 ms, even though STAI_TBS was not itself significant. The least squares mean difference between cTBS and iTBS was significant in the model without STAI_TBS. For the model including state anxiety, that difference was marginalized. TBS alone explained 3.1% of the variance in PTT (conditional variance: 65.8%) whereas the addition of STAI increased the explained variance to 7.7% (conditional variance: 60.18). See Table 2 for detailed LMER results.

4.3. Spontaneous breathing

4.3.1. RMSSD

The effect of iTBS on RMSSD during spontaneous breathing was significant (p = .009), however the effect of cTBS was not (p = .11). TBS alone explained 4.2% of the variance in RMSSD (conditional explained variance: 64.5%). Once included as a covariate, STAI_TBS predicted RMSSD (p = .02) and suppressed the significance of iTBS (p = .056). The suppression of the effect of iTBS appears to be meaningful, as adding STAI increased the marginal explained variance in RMSSD to 10.9% (conditional explained variance 74%). The difference between cTBS and iTBS was not reliable in either model. See Table 3 for LMER results.

4.3.2. PTTm

There was a significant effect of cTBS on mean pulse transit time (p = .01) and no significant effect of iTBS (p = .79), with a marginal effect size of 5.0% and conditional effect size of 67.5%. With the inclusion of STAI_TBS, the effect of cTBS increased (p = .007), whereas iTBS remained non-significant, as did STAI_TBS, although by including STAI_TBS the explained marginal variance increased to 7.95% (conditional explained variance: 63%). The difference between cTBS and iTBS was significant in both models, with higher PTT values for cTBS. In the model with STAI as a covariate, with the average PTT latency in response to cTBS increased from 16.7 ms to 18.7 ms. See Table 3 for detailed LMER results.

4.4. Correlational analyses

4.4.1. STAI_TBS vs. PTT

Follow-up Pearson's correlations (two-tailed) assessed the relationship between PTT and state anxiety in the period immediately after stimulation during Slow and Spontaneous Breathing for each stimulation condition.

4.4.2. Spontaneous Breathing

No significant association between PTT and STAI_TBS emerged in response to sham (r = −0.26 df = 18, p = .27). However, there was a significant inverse association between these variables for both cTBS (r = −0.49 df = 19, p = .026) and iTBS (r = −0.41 df = 22, p = .046).

4.4.3. Slow Breathing

PTT and STAI_TBS were not significantly correlated for sham (r = −0.2, df = 18, p = .39), whereas there were significant inverse associations between PTT and STAI for cTBS (r = −0.52, df = 18, p = .02) and iTBS (r = −0.494, df = 22, p = .015).

Table 2
LMERs testing fixed effects (FE) of TBS and TBS including state anxiety after stimulation (STAI_TBS) for the Slow Breathing condition. The rows labeled cTBS − iTBS represents the least squares means estimate of the difference between these conditions.

Slow Breathing		TBS					TBS + STAI_TBS				
		FE	SE	L-95	U-95	*p*-Val	FE	SE	L-95	U-95	*p*-Val
LF-HRV (log)	Intercept	8.43	0.16	8.1	8.7	< .0001	8.22	0.37	7.5	8.9	< .0001
	iTBS	0.28	0.126	0.04	0.53	**.03**	0.26	0.13	−0.008	0.53	.06
	cTBS	0.09	0.132	−0.16	0.34	.52	0.07	0.14	−0.20	0.33	.63
	cTBS – iTBS	−0.2	0.13	−0.45	0.06	.12	−0.19	0.13	−0.45	0.07	.14
	STAI_TBS						0.006	0.011	−0.01	0.02	.54
PTT	Intercept	357.6	7.48	343.6	371.6	< .0001	383.3	17.5	347.0	416.1	< .0001
	iTBS	0.56	6.4	−10.4	13	.93	4.1	6.9	−8.8	17.9	.56
	cTBS	13.7	6.8	0.98	26.4	**.052**	16.5	7.3	0.7	33.0	**.029**
	cTBS − iTBS	13.2	6.4	0.19	26.1	**.046**	12.4	6.7	−1.1	26.0	.07
	STAI_TBS						−0.81	0.50	−1.78	0.19	.11

Table 3
LMERs testing the fixed effects (FE) of TBS and TBS plus the covariate STAI_TBS for the Spontaneous Breathing condition. The rows cTBS − iTBS represents the least squares means estimate of the difference between cTBS and iTBS.

Spontaneous Breathing		TBS					TBS + STAI_TBS				
		FE	SE	L-95	U-95	*p*-Val	FE	SE	L-95	U-95	*p*-Val
RMSSD	Intercept	38.7	4.7	29.2	48.2	< .0001	15.5	11.2	−6.4	40.5	.17
	iTBS	11.2	4.1	3.6	19.1	**.009**	7.9	4.0	−0.02	16.3	.056
	cTBS	7.1	4.3	−1.7	16.1	.11	4.6	4.1	−3.98	13.5	.27
	cTBS − iTBS	−4.1	4.1	−12.4	4.2	.32	−3.3	3.8	−10.9	4.4	.39
	STAI_TBS						0.73	0.31	0.08	1.36	**.023**
PTT	Intercept	357.6	7.0	343.9	370.3	< .0001	377.2	16.3	342.6	406.8	< .0001
	iTBS	1.6	5.9	−13.0	12.6	.79	4.2	6.4	−8.5	16.4	.51
	cTBS	16.7	6.2	0.21	27.9	**.01**	18.7	6.5	5.85	32.9	**.007**
	cTBS − iTBS	15.1	5.9	3.4	26.8	**.013**	14.5	6.0	2.3	26.7	**.02**
	STAI_TBS						−0.61	0.46	−1.43	0.24	.19

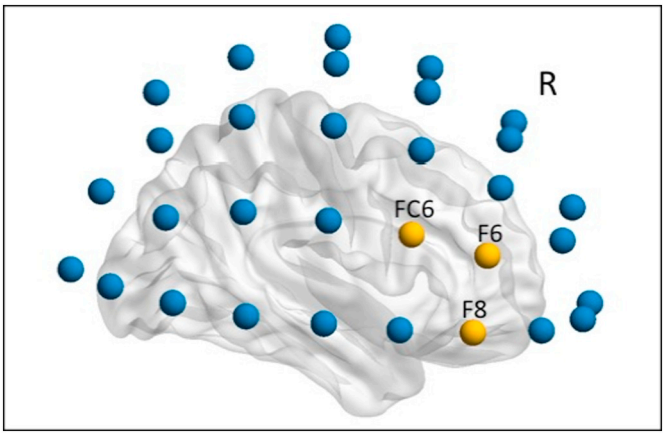

Fig. 1. EEG 10–20 electrode schematic for guiding placement of the stimulation coils. The wings of the coils overlapped FC6 and F8, while the center-top of the coil pointed to F6. The figure was rendered using BrainNetViewer using a template brain (Xia et al., 2013).

4.4.4. STAI_TBS vs. HRV

There were no significant simple correlations between state anxiety scores and RMSSD and LF-HRV.

5. Discussion

This randomized, sham-controlled repeated-measures study compared the effects of a single application of 600 pulses of intermittent, continuous, and sham theta-burst stimulation over the right frontotemporal cortex on resting state cardiovascular responses in healthy adults. We examined these effects under conditions of Spontaneous and Slow (0.1 Hz) Breathing. Furthermore, due to the potential for brain stimulation procedures to transiently increase anxiety in participants, resulting in an altered cardiovascular state, we determined whether stimulation-induced anxiety confounds cardiovascular responses to non-invasive brain stimulation. Consistent with our expectations that verum stimulation increases anxiety in participants, we found that verum iTBS and cTBS significantly increased state anxiety relative to sham TBS. The importance of controlling for stimulation-induced anxiety in studies of neurocardiac interactions was supported, and the influence of state anxiety as a covariate is discussed below in the context of each model.

For the stimulation effects, we observed significantly increased RMSSD after iTBS relative to sham during Spontaneous Breathing. Additionally, we observed significantly increased LF-HRV power during Slow Breathing after iTBS relative to sham. State anxiety was subsequently added as a covariate to determine whether the increase in anxiety during verum stimulation accounts for HRV responses to iTBS. Once added as a covariate, state anxiety attenuated the significance of

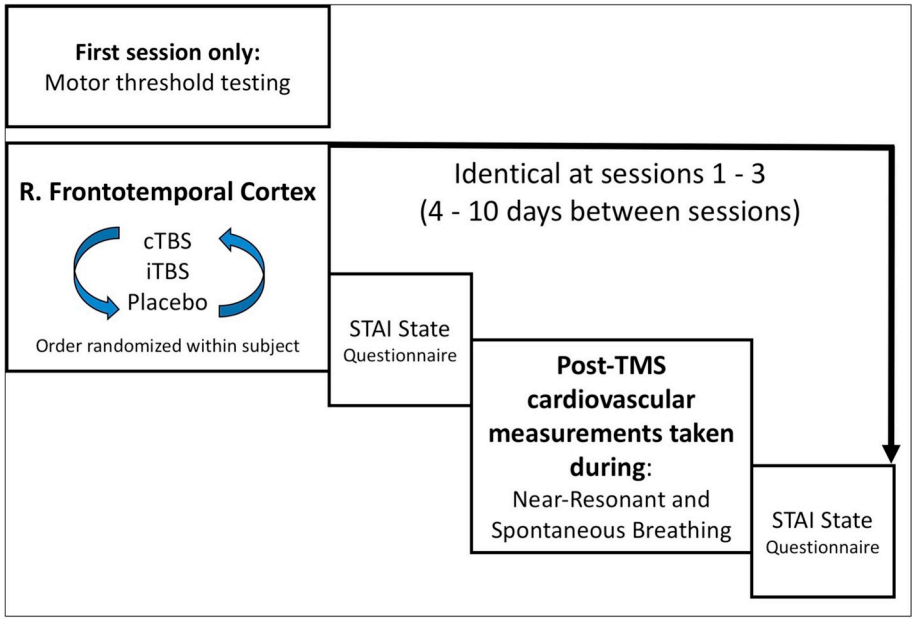

Fig. 2. Study design schematic. Participants came to the lab on three occasions during which they received Sham, cTBS and iTBS stimulation. The order in which stimulation protocols were applied was randomized for each participant. After stimulation, participants reported state anxiety, performed the breathing tasks, then reported state anxiety again.

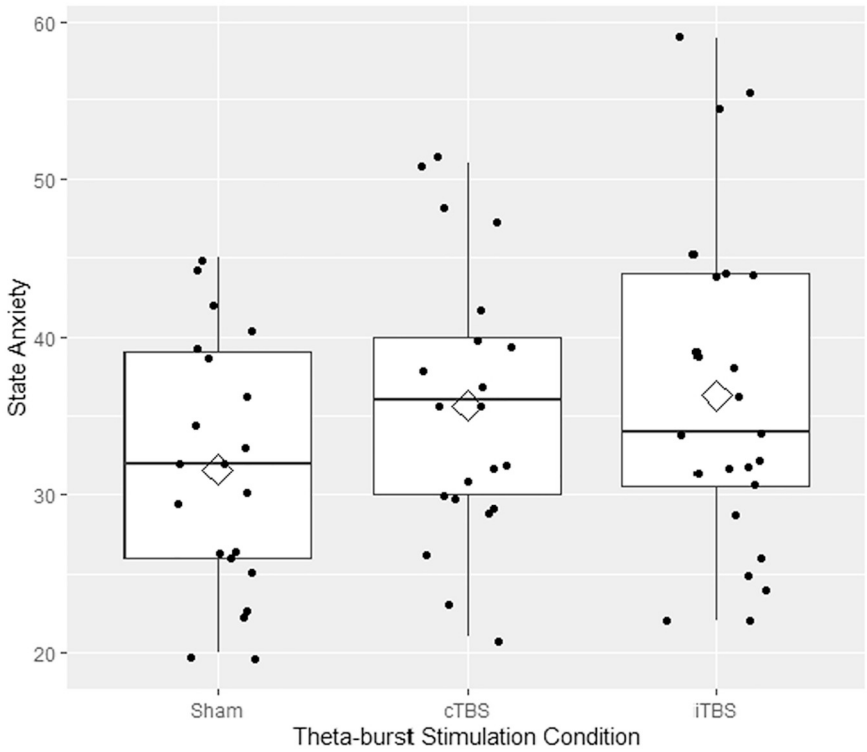

Fig. 3. State anxiety immediately after stimulation for each experimental condition (sham, continuous theta-burst (cTBS), and intermittent theta-burst (iTBS)). Empty diamonds reflect the condition mean. Data points are jittered to enhance visualization of individual scores.

iTBS on both RMSSD during Spontaneous Breathing and LF-HRV during Slow Breathing. For LF-HRV, the degree of attenuation was not meaningful, as state anxiety only explained an additional half percent of variance in LF-HRV during Slow Breathing exercise. Yet for RMSSD, the inclusion of state anxiety was significant, positive in sign (indicating higher RMSSD with greater anxiety) and resulted in iTBS becoming a non-significant predictor of RMSSD. The change appears to be meaningful, since state anxiety increased the marginal explained variance in RMSSD from 4.2% to 10.9%. The differential relevance of state anxiety for LF-HRV versus RMSSD is likely because RMSSD is considered a vagally-mediated measure of HRV, whereas LF-HRV during Slow Breathing largely reflects resonance processes between heart rate and blood pressure.

In contrast, cTBS resulted in a distinct pattern of effects on cardiovascular responses. Specifically, it elicited increased PTT latency relative to both iTBS and sham. When state anxiety was included as a covariate, it enhanced the strength of the effect of cTBS on PTT even though it was not statistically significant in either model (which may point to an issue of low power). Despite its non-significant *p*-value, including state anxiety as a covariate explained approximately twice or more variance in PTT than the models without across both breathing conditions. In the follow-up analysis of the correlation between PTT and state anxiety, we found that verum stimulation drove strong and significant inverse associations between PTT and state anxiety, whereas the association between PTT and anxiety was not significant after sham stimulation. These patterns of association suggest that PTT is sensitive to acute inductions of psychological stress and anxiety, given that verum stimulation was significantly more anxiety-provoking compared to sham. In other words, greater anxiety decreases PTT latency, whereas cTBS increases PTT latency. Adding state anxiety appears to have a potential to “unmask” the effect of cTBS on PTT.

These results contribute to evidence that TMS is effective at altering cardiovascular autonomic outflows in a “top-down” manner. However, estimates of these effects are impacted by stimulation-induced state anxiety. The potential influence of such confounds was raised in the meta-analyses by Makovac et al. (2017) and Schestatsky et al. (2013), and here we provide novel evidence supporting the relevance of this issue. For example, Makovac and colleagues reported only a small effect size for blood pressure reductions. In light of the present evidence that cTBS increases PTT latency, it is reasonable to speculate that controlling for confounds inversely associated with cardiovascular responses could increase observed effects. On the other hand, the reported effect sizes for vagally-mediated HRV may be lower if stimulation induced anxiety was accounted for. Schestatsky and colleagues' conclusions may have also been constrained by their inability to assess the influence of important covariates. Measuring stimulation-induced state anxiety or similar covariates during experiments would increase the precision of these estimates. It must be mentioned that these concerns are specific to experimental situations in which cardiovascular responses are measured concurrent or proximal to the stimulation. If studies were carried out such that cardiovascular changes were measured at a different time compared to stimulation, then the confound created by stimulation procedures should not be a concern. However, this approach is may be more easily applied in studies of clinical populations who are undergoing repeated brain stimulation treatments with an aim of long-term changes in symptoms.

We obtained a positively signed effect of state anxiety on RMSSD. The positive sign is notable since an inverse relationship between vagally-mediated HRV and negative affective states may be expected (Sloan et al., 2017). However, in healthy individuals, cardiac reactivity and subsequent cardiac vagal recovery are processes that may reflect adaptive responses to acute stressors (Balzarotti et al., 2017). Such a dynamic could produce positive associations between acute stress induction and HRV in experimental settings. The relationship between acute stress and cardiac vagal response is likely to have significant heterogeneity across individuals or populations, however. For instance, individuals with major depression show reduced cardiac reactivity and recovery in response to physiological and psychological stressors (Salomon et al., 2013).

It is also notable that iTBS and cTBS did not induce opposing effects

on cardiovascular responses given that these protocols are expected to have excitatory and inhibitory effects on cortical excitability, respectively. However, single session iTBS or cTBS may not exert opposing effects on prefrontal systems. Transcranial magnetic stimulation protocols (including i- and cTBS) facilitate GABAergic (ϒ-aminobutyric acid) and glutamatergic transmission, with complex effects on intracortical and cortico-limbic interactions (Baeken et al., 2017a). This is a complexity that is reflected in functional connectivity studies of cTBS and iTBS on prefrontal targets (Iwabuchi et al., 2017; Gratton et al., 2013).

With regards to possible neurofunctional pathways that may have mediated the observed effects of iTBS and cTBS on HRV and PTT, Pollatos et al. (2016) describe their stimulation heuristic as targeting the anterior insula. However, it is doubtful that the figure-of-eight coils can directly stimulate this region (Coll et al., 2017). Yet, the anterior insula may be stimulated transynaptically via the frontal operculum, which is plausible based on simulation studies of their heuristic (Coll et al., 2017; Pollatos et al., 2017). Direct electrical stimulation of the insula and operculum in humans also reveals strong reciprocal connectivity between these regions (Almashaikhi et al., 2014), an anatomical feature which is echoed in functional neuroimaging studies (Gratton et al., 2013). Additionally, cTBS of a left frontal operculum target caused the dorsolateral prefrontal cortex to become more tightly coupled with nodes in the default mode network, including the anterior cingulate (Gratton et al., 2013), which is a region that participates in visceromotor control through direct and indirect connections with the amygdala, hypothalamus, PAG, NST, and medullary autonomic nuclei (Silvani et al., 2016). Consequently, it may be sufficient to access visceromotor networks by stimulating frontotemporal targets, although identifying the cortical and subcortical changes that may mediate neurocardiac effects of TMS will require functional neuroimaging studies with concurrent cardiovascular measurement. It is also worth considering an alternative, but not necessarily mutually exclusive mechanism through which TMS could exert autonomic influences is through the cranial nerves which are stimulated during the delivery of magnetic pulses. In this case, afferent projections of the trigeminal nerves to the brainstem could also indirectly engage autonomic pathways (Colzato and Vonck, 2017; De Cicco et al., 2018).

If TMS can indirectly generate beneficial effects on cardiovascular function through brain plasticity, then there are direct clinical implications. Major depression, anxiety disorders, and chronic stress are independent risk factors for the development of cardiovascular disease (Steptoe and Kivimäki, 2013; Song et al., 2019). The prefrontal cortex and insula are essential to the regulation of the stress response and are implicated in depression and anxiety disorders (Baeken et al., 2017b; Simmons et al., 2011). Consequently, dysregulation of prefrontal and insula systems may - in the long-term - result in autonomic and HPA-axis dysfunction that contributes to the development of cardiovascular disease (Cogiamanian et al., 2010). However, TMS may be relevant to more than just psychiatric conditions if the aim of treatment is improved physiological stress regulation. For instance, multivariate cluster analysis of cardiovascular reactivity patterns to laboratory psychological stressors identifies older individuals at risk for the development of hypertension at a 5-year follow-up (Brindle et al., 2016). In such a context, TMS or TBS could be used as a repeated intervention with the aim of reducing maladaptive cardiovascular reactivity patterns by inducing plasticity in cortical circuits involved in stress regulation.

5.1. Limitations

Although we employed a previously published frontotemporal heuristic which is described as targeting the anterior insula that has been shown to modulate neural cardiovascular processing, we did not use structural MRI guided neuro-navigation, which would have helped to more precisely define the target of stimulation. Additionally, since the sample size was modest, non-significant findings may have been a consequence of Type-II error, and the potential for Type-I errors are also enhanced when samples are small. However, it should be considered that our sample size was over one-third larger than the average sample size in the 18 TMS studies included in the Makovac et al. meta-analysis. Only three of these 18 studies reported a greater sample size than that of the present study. Clearly, this area of research is hampered by the small sample sizes that currently characterize the literature. The field would greatly benefit from larger studies that can yield more robust estimate of TMS effects on cardiovascular function. Towards this end, an advantage of the present study is that we provide a basis for estimating power in future studies that employ mixed-effects models. Estimating power for mixed effects regression can be challenging as it requires the specification of multiple parameter values that can be difficult to determine without pilot data. Future studies with larger sample sizes will permit an assessment of the heterogeneity of responses to TBS (which may be best modeled using mixed-effects methods) since individuals may have a large degree of response variability to neurostimulation with multifactorial determinants (Ridding and Ziemann, 2010), including baseline cortical excitability or metabolism within a region or network (Salomons et al., 2014). Other limitations include the site and laterality of stimulation: since we only stimulated a single area on the right hemisphere, we cannot evaluate the effects of left-sided stimulation, or whether the medial prefrontal cortex, another area implicated in cardiovascular control, could produce similar outcomes to those observed here. As there may be several prefrontal targets that could exert top-down effects on the relevant subcortical networks, future studies could be optimized by incorporating information from combined neurostimulation-fMRI studies. Another limitation is that our analyses used a specific measure of psychological anxiety, although other measures of stimulation-induced arousal, anxiety or fear should also be investigated in covariate analyses to improve estimates of cardiovascular responses to TMS. Lastly, although we used proxy measures for baroreceptor sensitivity (LF-HRV during Slow Breathing) and beat-to-beat systolic blood pressure (PTT), estimation of cardiovascular variables and their dynamics would be improved by gold-standard non-invasive measurement, such as with continuous blood pressure monitors, and impedance cardiography.

6. Conclusion

This study provides preliminary evidence that cTBS to the right frontotemporal cortex increases pulse transit time latency, which may suggest a reduction of systolic blood pressure or cardiac pre-ejection period via inhibition of beta-adrenergic outputs. We also provide evidence that iTBS to the same region enhances HRV (both RMSSD during spontaneous breathing and LF-HRV during slow breathing). However, controlling for anxiety induced by stimulation attenuates the effect of iTBS on vagally mediated HRV (RMSSD). These findings emphasize that stress or arousal in response to the sensory components of the stimulation (e.g. noise, peripheral nerve stimulation, etc.) influence cardiovascular responses to TBS, but that the direction of these effects may depend on stimulation parameters, stimulation site, participant characteristics, and the physiological response system measured. TMS remains a promising approach for the study of cortical regulation of cardiovascular autonomic function. We discuss additional ideas for optimizing studies with the aim of characterizing TMS effects on cardiovascular function with greater precision. Such knowledge may contribute to the development of non-invasive brain stimulation protocols for the treatment of stress-linked maladaptive cardiovascular function.

Declaration of competing interest

None.

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