Biological Psychology 162 (2021) 108097

Contents lists available at ScienceDirect

Biological Psychology

journal homepage: www.elsevier.com/locate/biopsycho

Neuro-Cardiac-Guided TMS (NCG TMS): A replication and extension study

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ARTICLE INFO

Keywords:
TMS
Heart rate
Frontal-vagal
Dorsolateral prefrontal cortex
Target engagement
Personalized medicine

ABSTRACT

Neuro-Cardiac-Guided Transcranial Magnetic Stimulation (NCG-TMS) was studied for its potential to specifically target the frontal-vagal network. Previous research demonstrated that prefrontal stimulation led to significant heartrate slowing. We aimed to replicate these results in a larger sample and extend the findings to investigate dose-response relationships, reproducibility and stimulation frequency (10 Hz and intermittent theta burst (iTBS)). Data of forty-five healthy controls were analyzed, of which 28 received 10 Hz TMS (NCG-TMS) and 27 iTBS (NCG-iTBS; 10 received both protocols) at different stimulation sites according to the 10−20-EEG system. NCG-TMS yielded a relative heartrate deceleration at the F3/4 coil position replicating earlier studies. Both internal consistency and dose-response relationships were found. For NCG-iTBS adverse events were reported and topography for frontal-vagal activation was more lateralised relative to NCG-TMS. These results indicate that we were able to transsynaptically stimulate the frontal-vagal network and that excitability thresholds for the prefrontal cortex may differ relative to motor cortex.

1. Introduction

Despite the variety of available treatments for Major Depressive Disorder (MDD), up to 30–40 % of patients fail to achieve remission (Kessler and Bromet, 2013). Antidepressant medication is a first-line treatment (Anderson et al., 2008), but neuromodulation treatments such as repetitive transcranial magnetic stimulation (rTMS), transcranial direct current stimulation (tDCS), deep brain stimulation (DBS) and vagal nerve stimulation (VNS) also show promising clinical benefit in MDD (Brunoni et al., 2017a, 2017b; Donse et al., 2017; Mayberg et al., 2005; Rush et al., 2000; Schlaepfer et al., 2013). These treatments all target specific brain structures that are thought to be part of the depression network, such as the dorsolateral prefrontal cortex (DLPFC), the dorsomedial prefrontal cortex (DMPFC), the subgenual anterior cingulate cortex (sgACC) and vagus nerve (VN). Stimulation of these targets has been shown to result in symptom improvement in MDD (Downar et al., 2014; Downar and Daskalakis, 2013; Mayberg et al., 2005). The underlying mechanisms of these neuromodulation treatments, suggest altered network connectivity within the DLPFC, (sg)ACC and VN network which may be mediating clinical response (Fox et al., 2012; Liston et al., 2014). This frontal vagal network theory has been reviewed in detail by Iseger et al. (2019a), and provide the basis for a new targeting method for brain stimulation in depression (Iseger et al., 2017). In short, there is growing evidence of functional connectivity between these depression key nodes, and all of these nodes have been related to heart rate changes with neuromodulation. The vagus nerve especially is involved in parasympathetic signaling; stimulation of this nerve consequently leads to heart rate decelerations (Buschman et al., 2006), leading to believe that stimulation of the DLPFC and sgACC may also activate this parasympathetic signaling pathway. Moreover, MDD has been associated with higher heart rates, lower heart rate variability and higher risk for heart disease (Koenig et al., 2016), indicating a direct interplay between MDD and the frontal vagal network and a possible imbalance between parasympathetic and sympathetic activation.

Currently, several methods exist for localizing the DLPFC for TMS treatment without neuronavigation. The most common methods are the "5 cm standard procedure" and the "Beam-F3 method" (Beam et al., 2009). Both methods are based on measurement of physical aspects of

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https://doi.org/10.1016/j.biopsycho.2021.108097
Received 18 October 2020; Received in revised form 20 February 2021; Accepted 20 April 2021
Available online 22 April 2021

the skull. Although these possess some validity on the group-level, they are limited in their precision particularly when it comes to inter-individual variation of structural and functional connectivity (Fox et al., 2013; Mir-Moghtadaei et al., 2015). Neuronavigation methods using MRI do account for individual variation, but these methods are costlier and more time-consuming. Moreover, these methods navigate based on blood-oxygen-level-dependent (BOLD) signal or structural targets (e.g. Brodmann areas) and do not always consider functional connectivity. However, neuro-navigation methods using functional connectivity have been investigated, such as functional connectivity between the DLPFC and the sgACC, where the sgACC is used as a seed region to identify the appropriate prefrontal area target that shows the highest anti-correlation with the sgACC (Fox et al., 2012). Still, there is considerable inter-individual variation in functional connectivity patterns when assessed on different occasions (Ning et al., 2018), although developments have been made recently (Cash et al., 2021). The utility of DLPFC to sgACC functional connectivity in predicting clinical efficacy however, was further demonstrated by a prospective validation study (Weigand et al., 2018) and an independent validation study of these findings (Cash et al., 2019).

To provide a new and theoretically widely applicable approach for individually targeting rTMS, we recently proposed and tested another method for identifying the right cortical target for rTMS, based on studies showing heart rate decelerations after DLPFC-TMS (Makovac et al., 2016). In a pilot study, we have provided preliminary data for Neuro-Cardiac-Guided TMS (NCG-TMS) as a novel method for functionally targeting the interplay between the depression network and the heart-brain axis, or the frontal-vagal pathway (Iseger et al., 2017).

In this pilot study, several cortical brain regions of ten subjects were stimulated with 10 Hz rTMS trains. Relative to the primary motor cortex (C3/C4), pre-frontal stimulation at F3/F4 led to significant HR deceleration, with individual variation; for some subjects the relatively more posterior FC3/FC4 location led to the most pronounced HR decelerations instead of F3/F4, demonstrating the potential to use HR as a functional outcome measure reflecting engagement of the frontal-vagal network, and possibly allowing to individually target the depression network (Iseger et al., 2019a). For this method, small timeframes were used to determine heart rate deceleration. The rationale behind this is that stimulation of the vagus nerve usually results in an immediate response of the heart, typically occurring within the cardiac cycle in which the stimulation occurred and lasting only for about one or two heartbeats after stimulation. Return to a normal HR is very rapid after the activity of the vagus nerve is normalized (Hainsworth, 1995; Iseger et al., 2019a; Shaffer et al., 2014).

Recently, these results have been independently replicated by Kaur et al. (2020) in 20 healthy subjects. Subjects underwent left-sided NCG-TMS, and a significant larger heart rate deceleration was found for F3 compared to C3 (Kaur et al., 2020).

In order to further investigate and validate the NCG-TMS method as an applicable frontal-vagal target engagement measure, the following aspects need to be investigated: 1) reproducibility of the pilot data in a larger sample; 2) individual test-retest reliability; and 3) a dose-response relationship between rTMS intensity and HR deceleration.

The current study was set up in order to address these three points. The primary aim was to replicate the results from the pilot study and to further study laterality differences in a sample of initially 50 healthy volunteers. Dose-response relationships and test-retest reliability were also assessed. It was hypothesized that on the group level, F3/F4 and FC3/FC4 would lead to HR decelerations, similar to our previous findings (Iseger et al., 2017) and that most subjects would show maximum HR decelerations for these locations. A recent study indicated that the effects of intermittent Theta Burst Stimulation (iTBS) on heart rate were more pronounced (Iseger et al., 2019b), relative to the standard 10 Hz NCG-TMS protocol, therefore, we aimed to investigate NCG-TMS in the first 30 volunteers and NCG-iTBS in the second 30 volunteers (including 10 volunteers who received both NCG-TMS and NCG-iTBS).

2. Material and methods

2.1. Study design and participants

In our previous study (Iseger et al., 2017), stimulating the left hemisphere, location F3 was found to be the best location to cause a decrease in HR (total of three locations, n = 10, $d_{(F3\ vs\ C3)} = 1.01$), and in the right hemisphere the F4 location ($d_{(F4\ vs\ C4)} = 0.66$). A power calculation (GPower 3.1.9.2) was performed based on a 1-tailed *t*-test using two dependent means, with alpha at 0.05 and power at 0.90. For an effect size of 0.66 the required sample size was 22 (for each hemisphere), hence a sample size of 25 subjects per hemisphere (assuming a 10 % drop-out rate) was chosen, resulting in a total study sample of n = 50. This study was approved by the local Institutional Review Board (IRB, Utrecht University, Netherlands; NL63092.041.17) and registered on ClinicalTrial.gov (ID: NCT03652597).

Thus, data of 50 healthy subjects were collected (18–60 years). The standard exclusion criteria for TMS were followed; no history of epilepsy and no neurological conditions. Also, subjects who used psychotropic medication or medication for heart related issues, or with psychiatric disorders or cardiac conditions were excluded. Due to preliminary results indicating more profound effects of iTBS (Iseger et al., 2019b), it was decided after consultation with the IRB, to update the protocol to NCG-iTBS instead of 10 Hz NCG-TMS. Therefore, the healthy control sample consisted of a sample of 30 subjects using 10 Hz (NCG-TMS), and 30 subjects using iTBS stimulation (NCG-iTBS) (Fig. 1; ten subjects were tested with both 10 Hz and iTBS). Subjects were randomized to either the left or the right hemisphere and received the NCG-TMS assessment in session 1 while in session 2 dose-response relationships were explored. All participants provided written informed consent.

2.2. Procedures

Participants were randomized to NCG-TMS over the left or right hemisphere. For NCG-TMS, single 10 Hz trains of 5 s. each were applied to 8 different cortical 10-10 scalp locations on the left: F3, FC3, F1, F5, FC5, C3, FP1, AF3; or right hemisphere: F4, FC4, F2, F6, FC6, C4, FP2, AF4, with a Magstim Super Rapid^2 and a 70 mm figure-of-eight coil (The Magstim Company Ltd., Whitland, UK). Every location was stimulated 3 times in random order across all sites (inter-train-interval between two locations: 30 s). Specifically, every location was stimulated once, randomly, before moving on to the second and third random stimuli rounds. A custom EEG cap without electrodes (ANT Neuro) was used to locate the 10-10 system locations. Resting motor threshold (RMT) was determined prior to stimulation by using single pulse stimulation of the left motor cortex area, and visual observation of the thumb twitch. RMT was set as the minimum device intensity needed to obtain a motor response in 50 % of the applied pulses (Barker et al., 1985). Stimulation at all sites was applied at 100 % of the RMT. During stimulation, the participant was sitting in a relaxed upward position, was instructed to breathe normally and to avoid talking, since this could influence HR. The participant was asked to refrain from drugs and alcohol for 24 h as well as from caffeine and smoking for 2 h preceding the sessions. In session 2, the subject received 10 Hz trains on 2 different locations: the standard F3/F4 location, and their individual best NCG-TMS location, which was obtained from session 1. After again determining the RMT, the locations were stimulated at 70, 80, 90, 100 and 110 % RMT, 3 times at every intensity.

In addition, for NCG-iTBS, participants were randomized to NCG-iTBS over the left or right hemisphere. One minute of iTBS was delivered to 7 different cortical 10-10 scalp locations on the left: F3, FC3, F1, F5, FC5, C3, AF3; or right hemisphere: F4, FC4, F2, F6, FC6, C4, AF4, with a MagVenture MagPro R30 or a Deymed XT-100 both equipped with a 70 mm figure-of-eight coil, adopting the method from Iseger et al. (2019b), in where significant heart rate reductions were found in the first minute of iTBS stimulation (Iseger et al. 2019b).We chose to not

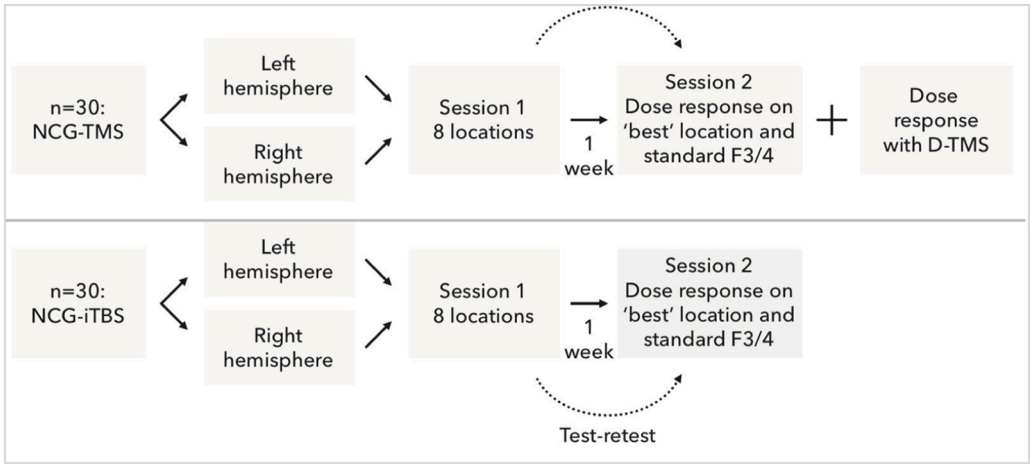

Fig. 1. Flow chart of the NCG-TMS study. Of 50 healthy volunteers, the first 30 were included for NCG-TMS. Every subject after that received iTBS (NCG-iTBS) instead of NCG-TMS. Ten subjects participated in both, the NCG-TMS trial and the NCG-iTBS trial, in order to compare inter-individual similarity, hence 30 subjects per trial. In Figs. 3 and 7, the Consort Flow Diagram shows the actual number of assessed participants.

apply iTBS to the FP1/2 site, since preliminary results did not show significant effects for this location and this is a very painful location, especially for the high intensity iTBS.

Between every location a resting period of 1−2 min was accommodated to allow the HR to stabilize. In session 2, the subject received 1 min of iTBS stimulation on 2 different locations: the standard F3/F4 location, and their individual best NCG-iTBS location which was obtained from session 1. After again determining the RMT, these locations were stimulated at 70, 80, 90, 100 and 110 % RMT for 1 min per location.

2.3. Physiological data acquisition

ECG data were co-registered in real-time with the TMS pulses and collected using the NCG-ENGAGE HR (neuroConn, Ilmenau, Germany) with a sampling rate of 1000 Hz. ECG was measured with three electrodes placed diagonally on the chest, with the ground electrode placed in the middle.

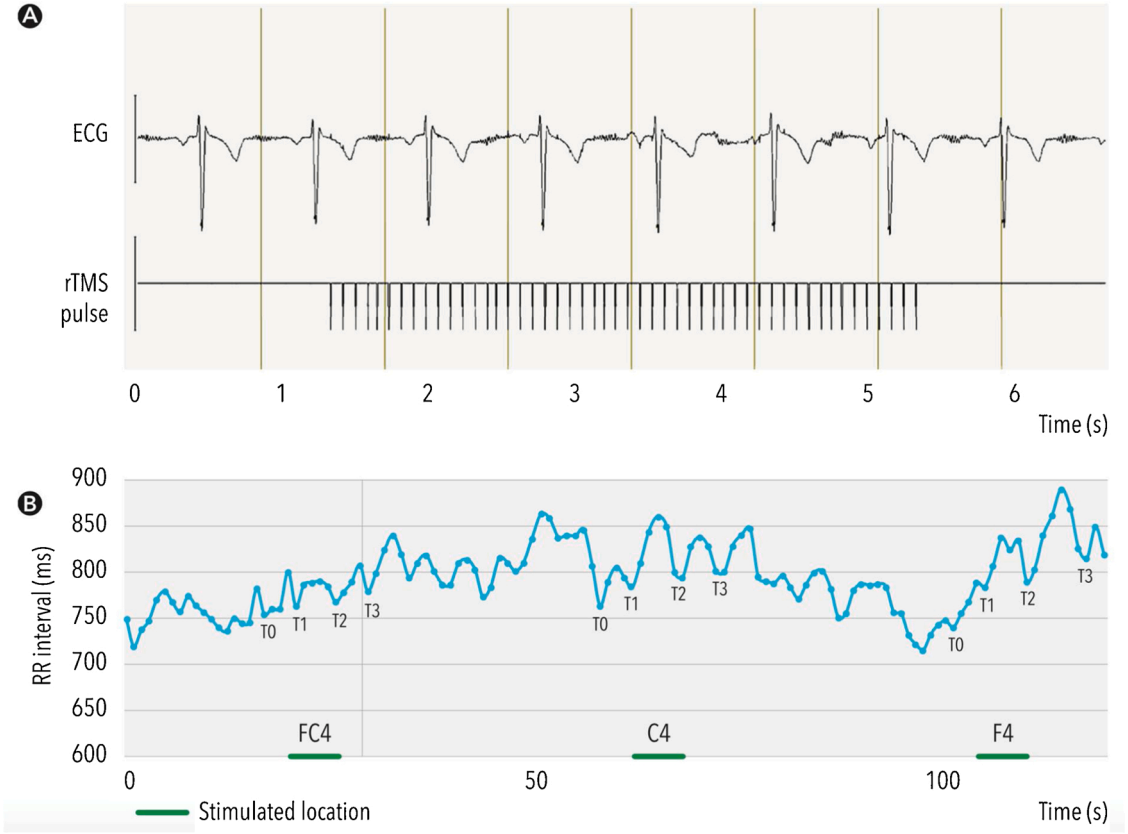

Fig. 2. Example of the NCG-TMS analysis method. ECG is recorded while simultaneously recording TMS stimulation pulses (2A). R-peaks from ECG where converted into an RR interval plot (2B). The troughs before stimulation where labelled as T0, whereas the 3 troughs after the start of stimulation where labelled as T1, T2, T3. Figure adapted from Iseger et al. (2017) Brain Stimulation.

2.4. Data processing

Data was processed similar to Iseger et al. (2017) but automated by the NCG-ENGAGE HR device. R-peaks within the ECG were scored and the interval between two R-peaks was calculated, creating RR interval data. Since breathing has a significant effect on HR, especially at short timeframes, only the troughs of the RR intervals were used, representing HR maxima. The pre-stimulation trough was labelled T0, and the first 3 troughs after the start of stimulation T1, T2 and T3 (see Fig. 2). In case of lower quality recordings where the NCG-TMS device could not label R-peaks correctly, R-peaks were manually scored when possible, using Brain Vision Analyzer (Brain Products), and further analyzed using customized Matlab scripts (The Mathworks), which were similar to the NCG ENGAGE HR.

Furthermore, for NCG-iTBS, only the slope of RR intervals across each minute of stimulation was calculated to provide a more simplistic measure of heart rate change. This was done using linear regression, method of least squares, in line with the approach used in Iseger et al. (2019b).

2.5. Statistical analysis

For NCG-TMS, RR intervals for the three trials per location were averaged and transformed into Z-scores (computed as $(T1\text{-}T0)/SD_{(T0)}$, where $SD_{(T0)}$ is the standard deviation of T0 across the three repeated stimulations for that location; same for T2 and T3). The normalization using $SD_{(T0)}$ was performed to reduce variance in effects of TMS due to individual differences and to the different timing for different locations. The Z-scores of T1-T3 were subsequently averaged and investigated for normality. The resulting Z-scores were evaluated on group-level, in order to investigate replication of Iseger et al. (2017).

As described in Section 2.1, 30 healthy controls were enrolled in each arm, i.e. NCG-TMS and NCG-iTBS. A recent review (Iseger et al., 2019a), presenting an individual participant data meta-analysis of 66 subjects, found no differences in left and right hemispheric NCG-TMS, thus, it was decided to combine the data of left and right hemisphere stimulated subjects to obtain enough statistical power. One tailed paired t-tests were used to test the primary hypothesis: stimulation at F3/4 leads to significantly larger HR decelerations relative to C3/4 (as found in our pilot study (Iseger et al., 2017)) and secondary: stimulation at FC3/4 leads to significantly larger HR deceleration relative to C3/4. Cohen's D effect sizes were calculated for the means between locations. All other sites were tested in an exploratory fashion and topographically plotted, but it was expected that on the group level, all would show HR accelerations rather than decelerations. This was assumed since the sensation of TMS (uncomfortable, sometimes painful, potentially stimulating surrounding muscles), would rather result in HR accelerations (sympathetic activation) instead of decelerations, especially in the TMS naïve healthy control group.

Test-retest reliability was tested by correlating RR interval change at the F3/F4 locations from session 1 to session 2 (at 100 % RMT), as well as paired t-tests (two-tailed). Additionally, Intraclass Correlation Coefficient (ICC) was obtained by running reliability analysis. Two-tailed dose-response relationships for HR deceleration were tested by correlating stimulation intensity expressed as a) percentage MT and b) as percentage stimulator output.

3. Results

3.1. NCG-TMS

3.1.1. Subject characteristics

For NCG-TMS, data from 30 healthy control subjects were collected. In total, 28 subjects were included for analyses, due to two dropouts prior testing (12 subjects left -, 16 right hemisphere stimulation; mean age: 31.0 ± 6.68 years, 12 males) see Table 1 and Fig. 3. Two subjects did not complete session 2. No side effects or adverse events were reported.

Table 1
Subject characteristics of the NCG-TMS and the NCG-iTBS group. Shown are the number of males (N male) as absolute number and percentage of total, age in years (y) (mean [SD]), motor threshold (mean [SD]) for session one and two, head size in centimeters (cm) (mean [SD]) and nasion-inion distance in centimeters (cm) (mean [SD]). For both groups, motor thresholds were not statistically different between sessions.

	NCG-TMS (n = 28)	NCG-iTBS (n = 27)
N male	12 (43 %)	8 (30 %)
Age (y)	31.04 (8.68)	31.78 (10.36)
RMT session 1	63.43 (8.73)	47.70 (6.43)
RMT session 2	62.31 (9.41)	45.00 (6.05)
Headsize (cm)	56.45 (2.17)	56.16 (1.58)
Nasion-Inion (cm)	35.20 (1.58)	34.87 (1.33)

3.1.2. NCG-TMS replication and extension

We found a significantly larger HR deceleration for F3/F4 compared to C3/C4 ($t(27) = 2.18$, $p = .038$, $d = .463$) and for FC3/FC4 compared to C3/C4 ($t(27) = 1.90$, $p = .069$, $d = .487$) with one-tailed t-tests. Post-hoc analysis with location as within-subjects factor and hemisphere as between-subjects factor, indicated no differences between hemispheres (F3/F4-C3/C4: $p = .561$; FC3/FC4-C3/C4: $p = .941$). All other locations showed HR accelerations as can be seen in Fig. 4. The spatial distribution can be found in Fig. 9A (with all data collapsed over one hemisphere for illustrative purposes).

There was an equal number of subjects showing the largest HR deceleration for F3/F4 (18 %), as to F1/F2, indicating inter-individual variation for optimal target sites, also in agreement with our pilot results.

3.1.3. Dose-response relationship

RR interval lengthening was observed to be correlated with *absolute* stimulation intensity values at the F3/4 region, ($r(129) = .297$, $p = .001$), explaining 7,56 % of the variance (Fig. 5). This shows that the higher the TMS intensity, the higher the RR interval lengthening. There was no significant effect of stimulation intensity (expressed as percentage resting motor threshold (%RMT)) on RR interval lengthening ($r(129) = .092$, $p = .299$).

3.1.4. Test-retest reliability

In order to assess test-retest reliability, z-scores from session 1 were correlated with z-scores at session 2. This was tested for F3/F4, since this location was available for every subject and both assessments. A significant correlation of $r(25) = .475$ ($p = .014$) was observed, explaining 23 % of the variance, thus indicating internal consistency (Fig. 6). A paired sample *t*-test indicated that there were no differences in the amount of HR deceleration ($t(25) = .86$, $p = .399$). Additionally, 46.43 % of the subjects expressed HR decelerations during session 1, while 42.31 % of the subjects expressed HR decelerations during session 2, with an overlap of 73 % suggesting sound stability on the individual level. Reliability analysis resulted in an intraclass correlation coefficient (ICC) of .527.

3.2. NCG-iTBS

3.2.1. Subject characteristics

Thirty subjects were included, and data from 27 subjects were collected (3 subjects cancelled their participation prior testing), of which 14 were allocated to stimulation in the right- and 13 to stimulation in the left hemisphere (mean age: 31.78 ± 10.36 yrs, 8 males), see Table 1 and Fig. 7. One subject experienced an adverse reaction to all stimulation sites (thus data were excluded from the primary analysis), and 4 subjects experienced adverse reactions to the exploratory sites

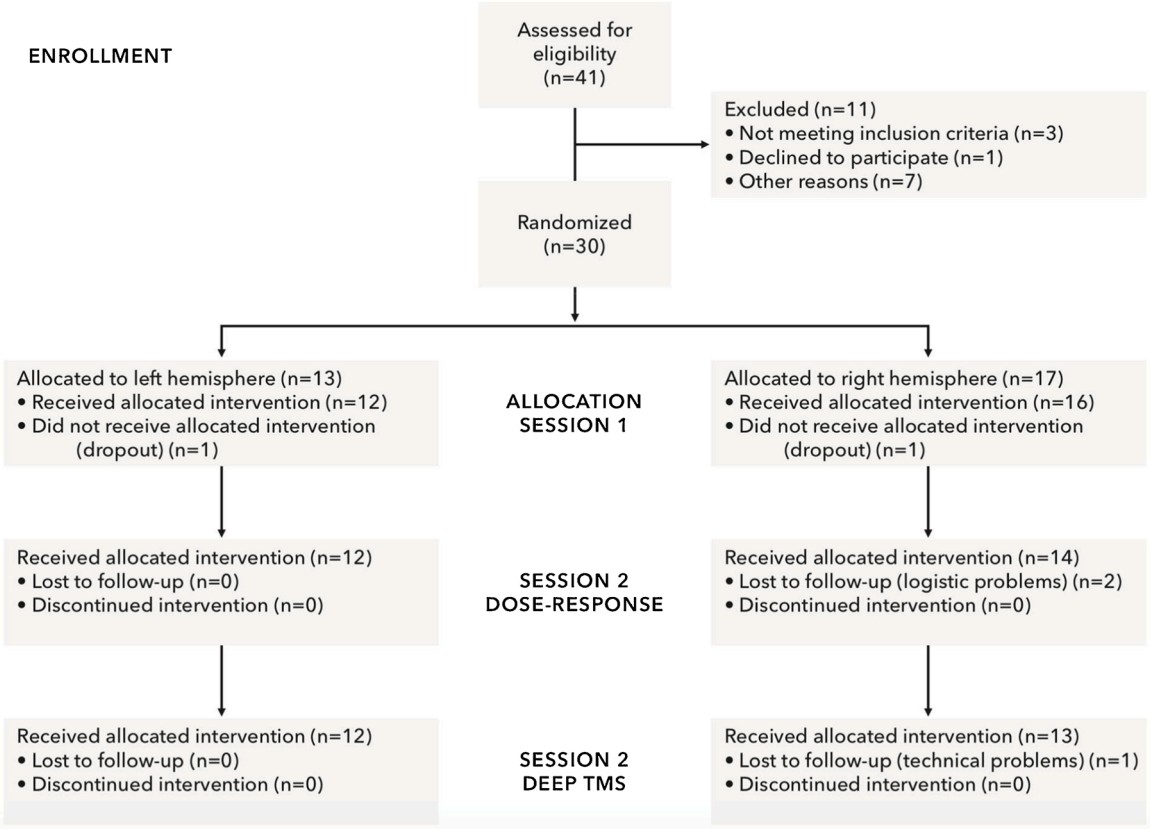

Fig. 3. CONSORT Flow diagram of the NCG-TMS study.

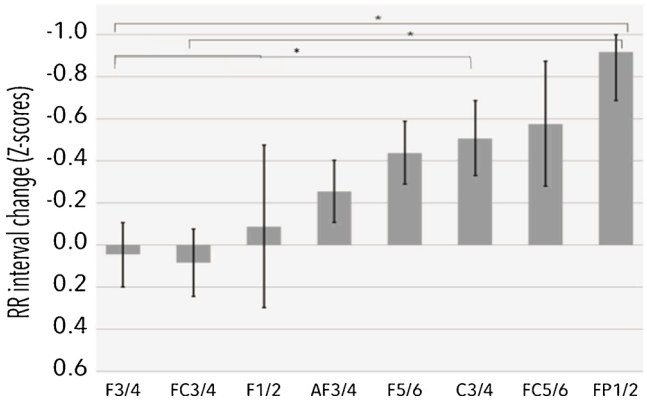

Fig. 4. Group level Z-scores of RR interval changes. The larger the Z-score, the larger the RR interval change (equaling HR deceleration). Note that the Y-axis is inversed in order to represent HR increases as an upward bar and HR decreases as a downward bar. Only for F3/4 and FC3/4 HR decelerations were observed, whereas all other sites show accelerations (A * indicates a p-value below 0.05 (one-tailed)).

(thus were excluded from the exploratory analysis, and consequently did not participate in session 2). Adverse reactions that were reported were lightheadedness (1), emotional reactions, i.e., crying (1) and painfulness (3). No serious adverse events were reported. For the second session, 2 subjects cancelled the appointment due to scheduling constraints. One subject did complete the second session, but the data were of bad quality (r-peaks could not be scored adequately), therefore excluded from analysis, leaving 19 subjects for analysis of session 2.

3.2.2. NCG-iTBS

There was no statistically significant difference for F3/F4 compared to C3/C4 ($t(25) = 1.65$, $p = .112$, $d = .266$), and for FC3/FC4 compared to C3/C4 ($t(25) = 1.62$, $p = .118$, $d = .218$) in 26 subjects (see Fig. 8). Post-hoc analysis with location as within-subjects factor and hemisphere as between-subjects factor, indicated no differences between hemispheres. When including all other tested locations, there was a significant effect of location ($F(6, 16) = 3.84$, $p = .014$), but this was due to unexpected large heart rate decelerations at other locations, namely FC5/6 and F5/6 (see Figs. 8 and 9B). The FC5/6 location differed significantly from F3/4 ($p = .001$), FC3/4 ($p = .001$), C3/4 ($p = .001$), F1/2 ($p = .001$), AF3/4 ($p = .004$) and F5/6 differed significantly from F3/4 ($p = .002$), FC3/4 ($p = .003$), C3/4 ($p = .001$), F1/2 ($p < .001$), AF3/4 ($p = .001$).

Most subjects were showing the largest HR deceleration for FC5/FC6 (11) followed by F5/6 (4) and for F3/4 only 2 subjects, indicating inter-individual variation for optimal target sites (sites with 1 or less subjects are not mentioned).

The results using 1 min. of iTBS stimulation yielded both on the group level, as well on a within-subject comparison in ten subjects (where NCG-TMS and NCG-iTBS were both applied), more lateralized sites that demonstrated the clearest HR deceleration (FC5/6 and F5/6), also see Fig. 9.

3.2.3. Dose-response relationship

Analyses was corrected for machine type (partial correlation), since a different TMS device was used.

There was no significant effect of %RMT on RR interval lengthening during stimulation on the F3/4 location (neither with repeated measures ANOVA nor with correlation analysis ($r(18)=-.004$, $p = .968$)). Additionally, no significant correlation was observed when using absolute stimulation intensity values rather than %RMT ($n = 19$, $r = .137$, $p = .193$; Fig. 10A). However, since F3/4 are not the 'best locations' on

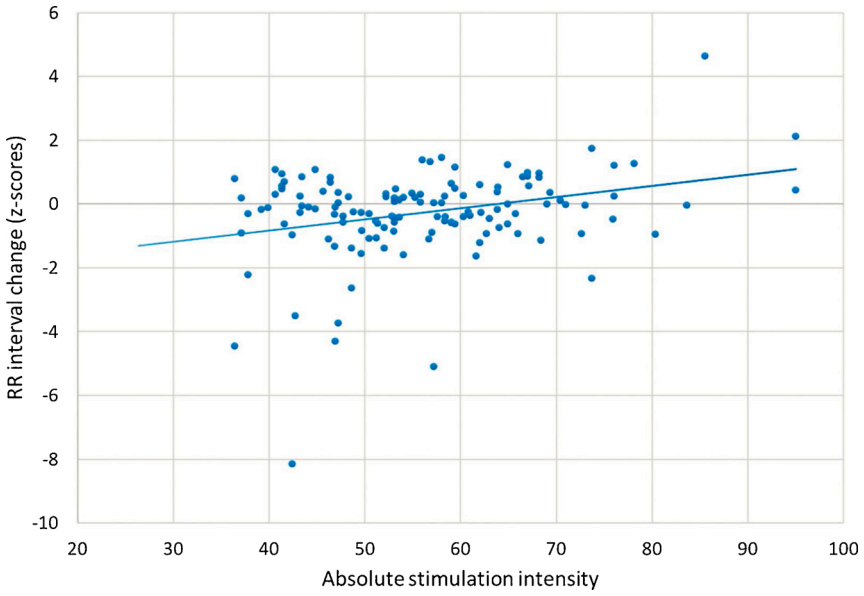

Fig. 5. Correlation plot between RR interval change in z-scores and TMS intensity, during stimulation of the F3/4 region. The blue dots represent 5 stimulation intensities for each individual subject (n = 26, r(129) = .297, p = .001, r^2 = 0.17) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

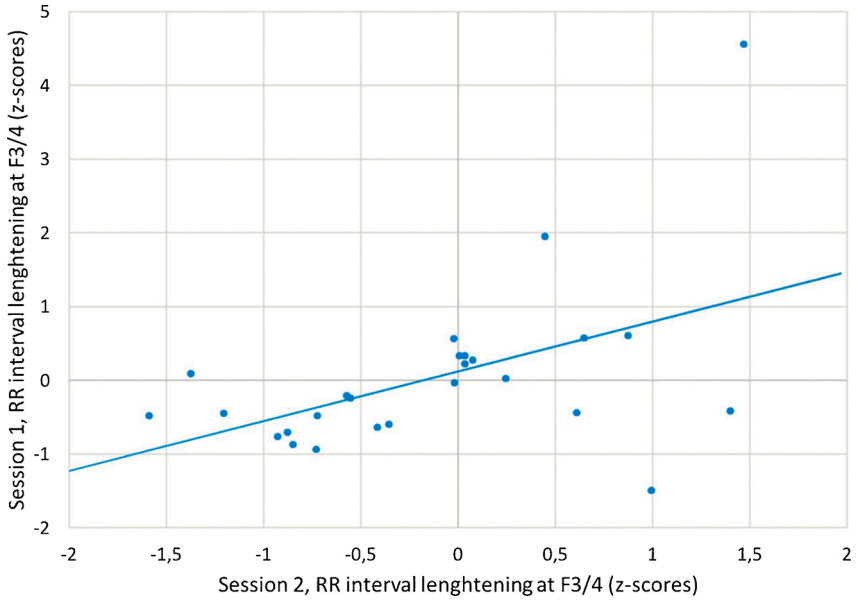

Fig. 6. Correlation plot between RR interval change in session 1 with RR interval change in session 2, during stimulation of the F3/4 region (r = .475, p = .014, r^2 = 0.23).

group level (only for 9 % as indicated above), correlation analysis was also performed for the 'individual best location' and in particular for subjects for who FC5/6 was the best location.

For the individual best location, there was no correlation with %RMT (r(18) = .009, p = .929), but there was a significant correlation with absolute stimulation intensity (r(91) = .206, p = .048). We suggest this was driven by the individuals with FC5/6 as best location, since when selecting on these individuals, an increased significant correlation was found with absolute stimulation intensity (r(51) = .374, p = .006, Fig. 10B) as well.

3.2.4. Test-retest reliability

In order to assess test-retest reliability, RR interval lengthening at session 1 was correlated with RR interval lengthening at session 2. This was tested for F3/F4, since this location was available for every subject and both assessments. No significant correlation was observed (r(18) = .257, p = .288). Reliability analysis resulted in an intraclass correlation coefficient (ICC) of .240, indicating no internal consistency.

However, since F3/4 are not the 'best locations' on group level, correlation analysis was also performed for only subjects for who FC5/6 was the best location. This resulted in a significant correlation between session 1 and 2 (r(10) = .720, p = .012, Fig. 11) and a high ICC score of .720. However, paired t-tests did indicate differences in the amount of heart rate deceleration that was reached per session (t(10) = 3.184, p = .010), showing smaller heart rate decelerations during session 2. This may be explained by the fact that individual RMT's were in general lower during session 2 (p = .022). When controlling for RMT differences, correlation between session 1 and 2 did not change.

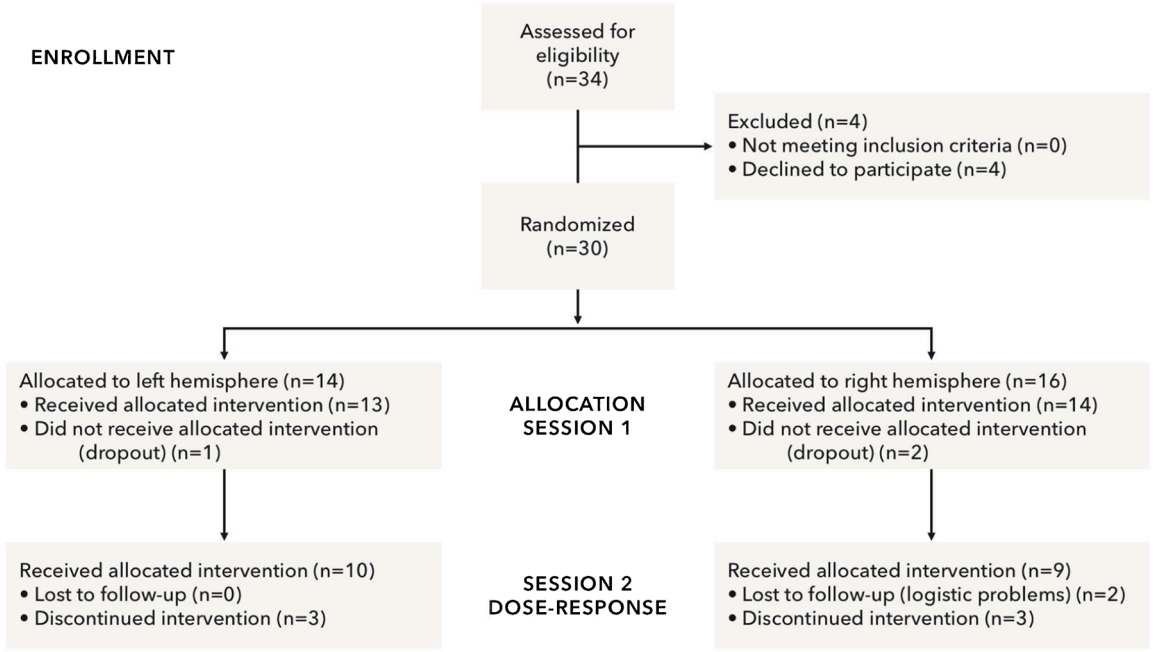

Fig. 7. CONSORT Flow diagram of the NCG-iTBS healthy controls study.

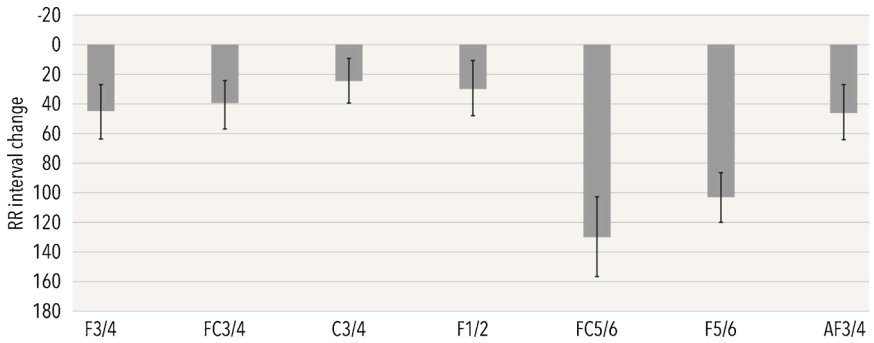

Fig. 8. RR interval changes for the NCG-iTBS group. The larger the bar, the larger the RR interval change (equaling HR deceleration). Note that the Y-axis is inversed in order to represent HR increases as an upward bar and HR decreases as a downward bar, and significant differences are not depicted in this figure. Also, the RR interval change is not comparable to the change depicted in Fig. 4, since different quantification methods were used.

4. Discussion

Validation of the NCG-TMS approach required replication, assessment of test-retest reliability and esthablishing a dose response relationship. Here, we present results that replicate our earlier findings, supporting the validity of the NCG-TMS approach to activate the frontal-vagal pathway (using 10 Hz rTMS trains). We show that, on the group level, the largest HR decelerations were found at F3/F4 and FC3/FC4. Furthermore, this method demonstrated sound test-retest reliability, and a dose-response relationship between HR effects and stimulation intensity as defined by % maximum machine output, but not by %RMT. The results for NCG-iTBS demonstrated more pronounced HR decelerations, albeit with a different topography requiring further research on the underlying functional neuroanatomy.

Similar to Iseger et al. (2017) and Kaur et al. (2020), HR decelerations were found for F3/F4 and FC3/FC4 on the group-level for the healthy control study. All of the other tested locations show HR accelerations rather than decelerations. In this study, left and right hemisphere conditions were merged in order to obtain adequate statistical power. Post-hoc analyses yielded no significant differences between hemispheres, confirmed by results from an individual participant data meta-analysis with adequate statistical power combining individual participant NCG-TMS data from 4 studies (total of 66 participants), which demonstrated no laterality effects (Iseger et al., 2019a). The fact that rTMS of both hemispheres had similar effects is also in line with recent studies questioning current hypotheses of hemispheric laterality in MDD. For example, Kovel et al. conducted MRI with laterality of thickness and surface area measures, but did not observe any specific laterality differences in MDD as compared to controls, as measured with MRI (de Kovel et al., 2019). In a large meta-analysis of EEG studies, van der Vinne et al. (2017) did not detect a significant difference in frontal EEG alpha assymetry between MDD and controls (van der Vinne et al., 2017). The finding that HR decelerations were only observed for F3/F4 and FC3/FC4 suggests transsynaptic activation of frontal-vagal pathways by prefrontal rTMS and supports further development of NCG-TMS towards an efficient method for assessing frontal-vagal 'target engagement'.

A recent study investigating cardiovascular differences between iTBS and sham stimulation showed that iTBS aimed at the Beam-F3 site had a significant effect on heart rate, blood pressure and several HRV

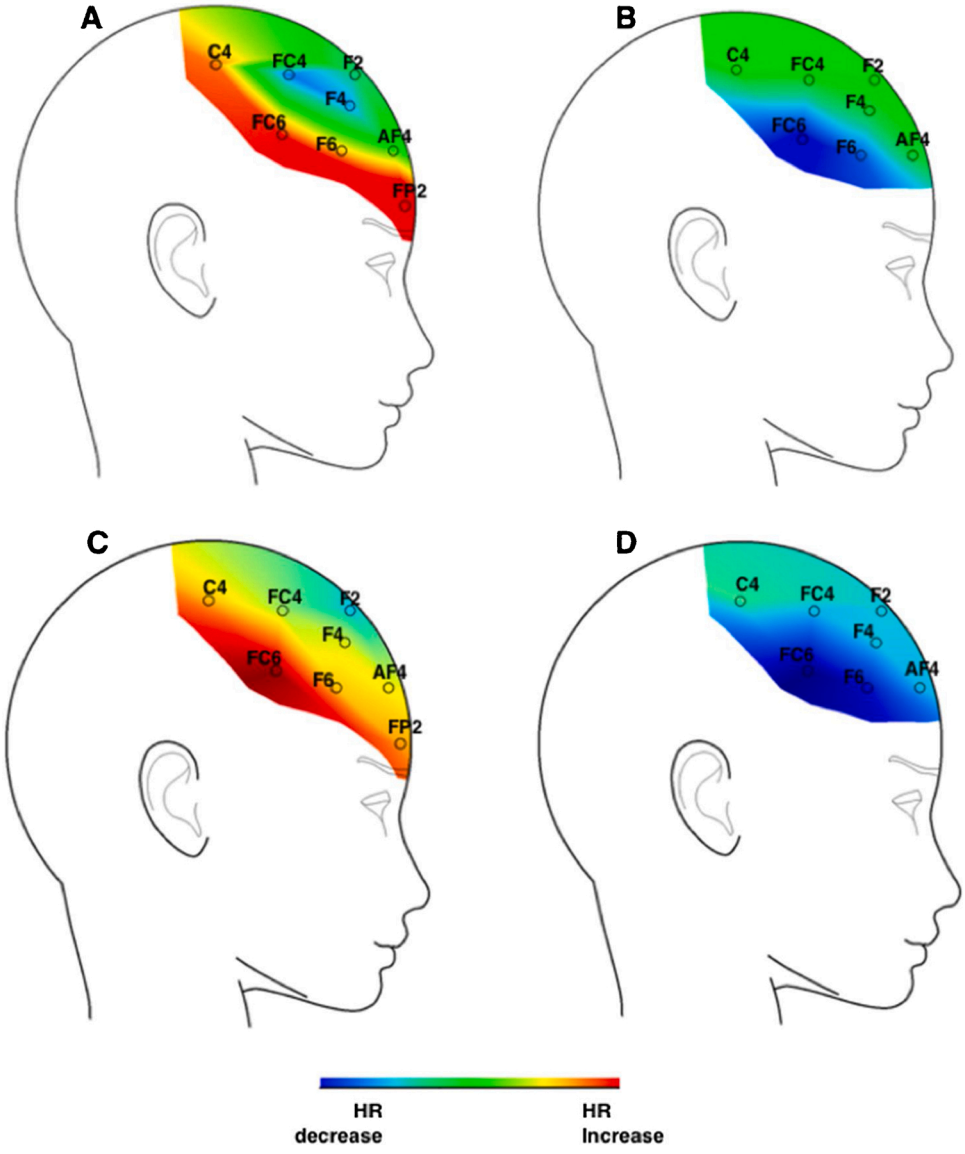

Fig. 9. Group level topographical plots of RR interval changes for the NCG-TMS treatment arm (A; visualization of Fig. 4), and the NCG-iTBS treatment arm (B; visualization of Fig. 8). Figure C and D represent the topographical plots of the subsample (n = 10) that received both NCG-TMS (C) and NCG-iTBS (D). The scale represents the inversed z-scores, blue indicates HR deceleration, orange/red indicates HR acceleration. All data was collapsed over one hemisphere for illustrative purposes. Note that only the indicated sites were stimulated and the color schemes in between sites are interpolated (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

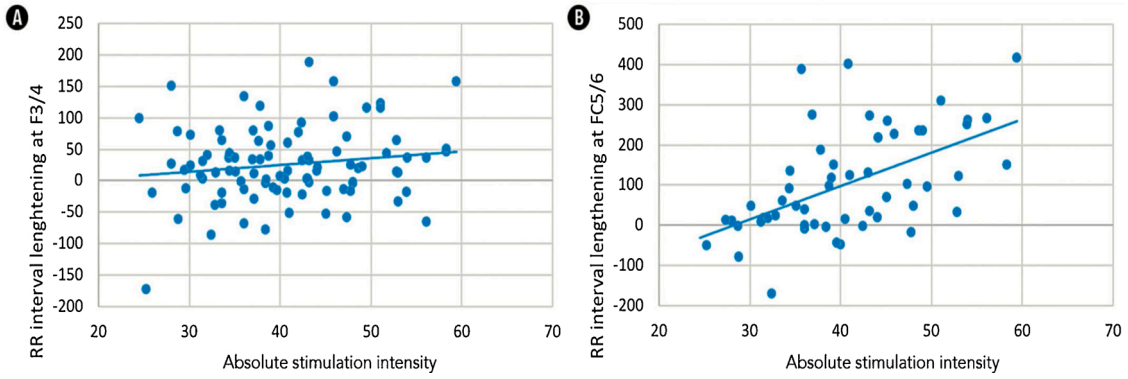

Fig. 10. Correlation plot between RR interval change and TMS intensity for the F3/4 location (A). The blue dots represent the 5 stimulation intensities for each individual subject (n = 19, r(91) = .137, p = .193, r^2 = .037). In B, the correlation plot between RR interval change and TMS intensity for each individuals with FC5 or FC6 as best location is depicted. The blue dots represent the 5 stimulation intensities for each individual subject (n = 11, r(51) = .374, p = .006, r^2 = .14) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

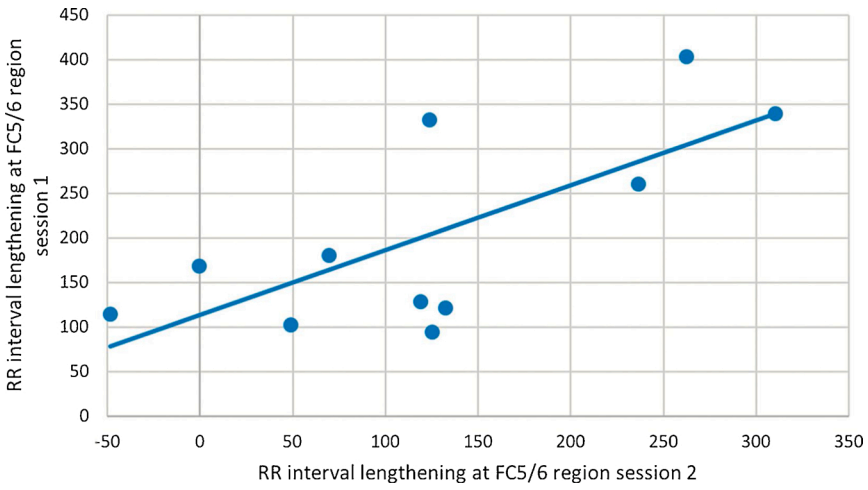

Fig. 11. Correlation plot between RR interval change in session 1 with RR interval change in session 2, during stimulation of the FC5/6 region ($r(10) = .720$, $p = .012$, $r^2 = .519$).

measures of short duration (Iseger et al., 2019b). The impact on HR seemed more pronounced than the HR effects with regular TMS. However, the results from NCG-iTBS in the current study requires further study and replication; the more pronounced HR effects may be attributed to the time-period in which HR decelerations were analyzed. For example, for iTBS this was a fixed timeframe of 1 min, while for 10 Hz, these were variable timeframes of around 20 s, while taking only the troughs of the RR interval. Thus, the NCG-TMS and NCG-iTBS may not be completely comparable. Also, due to methodological and scheduling reasons, we needed to resort to different types of equipment, which is a limitation of the study.

We demonstrated sound test-retest reliability for the NCG-TMS and NCG-iTBS methods. Furthermore, a dose response relationship was only detected for absolute machine output intensity, but *not* for stimulation intensity relative to the individual RMT. This suggests that excitability thresholds for prefrontal areas may differ from those for motor regions, and NCG-TMS may be instrumental in establishing an individual prefrontal excitability threshold. Such a difference between cortical areas has also been shown using motor and phosphene thresholds and may be due to differences in cortical structure and excitability between motor and non-motor regions (Gerwig et al., 2003; Stewart et al., 2001). Unfortunately, dose response relationships were not assessed for the other stimulation sites, which otherwise may have given information about the relationship between target location specificity and stimulation intensity.

A further question that deserves attention, is whether stimulation sites detected with the NCG-TMS method will eventually lead to a superior clinical outcome in MDD. Indeed, preliminary results in a small sample demonstrated that the amount of HR deceleration on F3 before treatment was correlated with HRSD reduction post-treatment (Iseger et al., 2019b). Future studies need to systematically assess the potential of NCG-TMS for individualizing treatment for MDD and other psychiatric disorders. Furthermore, it is not known whether NCG-TMS will normalize HR and HRV. Previous studies show that depression treatments generally do not normalize HRV, however one study on rTMS did show increased HRV after treatment (Udupa et al., 2011). It is possible that improved targeting may also impact heart rate in the long term.

It is of note, that not all subjects show HR deceleration to the same extent. TMS gives an unpleasant sensation, sometimes painful, which consequently may lead to sympathetic activation and thus overrule parasympathetic activation that would normally result in HR deceleration, since pain activates the sympathetic nervous system (Awad et al., 2001). Thus, a sympathetic:parasympathetic balance may partially explain the inter-individual variation of effects. As such, the degree of HR change may not be informative without a comparison to other (control) locations. Thus, an important notion deserving further study is that not the target site showing an HR deceleration may be the most effective location, but the site showing the *least* HR acceleration within such a sympathetic:parasympathetic balance. Future studies may include a sham condition or score pain sensations at each cortical stimulation site. Furthermore, patients already receiving TMS may already have habituated to stimulation, possibly leading to a diminished sympathetic response. In such a group of subjects, the parasympathetic response on heart rate might present itself clearer. However, we found no indication of such an effect over time during iTBS treatment (Iseger et al., 2019b).

The fact that after NCG-iTBS HR deceleration was detected at the more lateral sites FC5/6 and F5/6 was unexpected but raises some interesting hypotheses. For example, using cTBS, Pollatos et al. (2016) stimulated a region located between FC6-F6-F8, which is just beneath our stimulated location, targeting the insular cortex. The group investigated heart beat evoked potentials and found reduced amplitude of these potentials with cTBS. Another hypothesis is that not the insular cortex is stimulated, but the trigeminal nerve. Stimulation of the trigeminal nerve has been used to treat MDD (Cook et al., 2016) and has also been associated with HR deceleration (Meuwly et al., 2015). This makes sense, since stimulation on both FC5/6 and F5/6 often leads to muscle activity in the jaw, which can be a result of trigeminal nerve stimulation. Irrespective of which explanation is true, it is a fact that 10 Hz rTMS at optimal sites for iTBS did not lead to HR deceleration, indicating that this may be a frequency specific effect. In this respect, it needs to be emphasized that intensity response relationships may differ between 10 Hz rTMS (usually applied at $\geq$100 % RMT intensity) and iTBS (originally applied at 80 % active MT). In case the intensity response relationship would e.g. follow an inverted U shape, optimal stimulation sites may be localized at different spheres of the magnetic field for 10 Hz rTMS and iTBS both applied at the same intensity. Since our results are not conclusive, NCG-iTBS is currently not recommended for targeting the frontal-vagal network in clinical practice and requires further research. In order to investigate such a frequency dependent effect, it may be useful to study other stimulation protocols as well, including inhibitory protocols, such as 1 Hz rTMS, which was recently investigated by Kaur et al. (2020). Although looking at mean RR and not RR change, they found significantly lower RR intervals during stimulation at F3/4, compared to C3/4, suggesting low frequency rTMS affects heart rate in the opposite direction, in line with previous research reporting different biological effects between rTMS types (Fitzgerald et al., 2006). The exact mechanisms are not understood, but may be caused by low frequency rTMS causing a dampening of parasympatho-inhibition, allowing sympatho-excitation to increase

(Kaur et al., 2020).

5. Conclusions

To conclude, we successfully replicated our previous results, demonstrating that NCG-TMS with 10 Hz rTMS activates a frontal-vagal pathway resulting in HR deceleration, in a site-specific manner. These data confirm that the NCG-TMS method activates the frontal-vagal network with a potential use for individualizing rTMS treatment in MDD. Furthermore, our results indicate that excitability thresholds for prefrontal and motor cortex regions differ, and NCG-TMS may also be used for establishing prefrontal excitability thresholds.

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosures

MA is unpaid research director of the non-profit Brainclinics Foundation, a minority shareholder in neuroCare Group (Munich, Germany), and a co-inventor on 4 patent applications (A61B5/0402; US2007/0299323, A1; WO2010/139361 A1) related to EEG, neuromodulation and psychophysiology, but does not own these nor receives any proceeds related to these patents; Research Institute Brainclinics received funding from Brain Resource (Sydney, Australia) and neuroCare Group (Munich, Germany), and equipment support from Brainsway, Deymed, neuroConn and MagVenture. TI and MA are coinventors on a patent covering the Neuro-Cardiac-Guided TMS method (owned by neuroCare Group), but receive no proceeds or royalties. FP is a member of the European Scientific Advisory Board of Brainsway Inc., Jerusalem, Israel, and has received speaker's honoraria from Mag&More GmbH (Munich, Germany). His lab has received support with equipment from neuroConn (Ilmenau, Germany), Mag&More GmbH and Brainsway Inc., Jerusalem, Israel.

Acknowledgements

We would like to acknowledge neuroConn, in particular Klaus Schellhorn, who developed and provided us with the NCG-ENGAGE HR and for the development of the algorithms and Brainsway for providing the dTMS hardware used in the study. Finally, we acknowledge the support of Fiamma Cavallero, in the data-collection of healthy controls, and our clinicians, in particular, Rosalinde van Ruth, Vera Kruiver, Myrthe van Eerdt, and Joris van Neijenhof for their support in collecting data from the patient sample.

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