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Investigating high- and low-frequency neuro-cardiac-guided TMS for probing the frontal vagal pathway

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ABSTRACT

Background: Investigating approaches for determining a functionally meaningful dorsolateral prefrontal cortex (DLPFC) stimulation site is imperative for optimising repetitive transcranial magnetic stimulation (rTMS) response rates for treatment-resistant depression. One proposed approach is neuro-cardiac-guided rTMS (NCG-TMS) in which high frequency rTMS is applied to the DLPFC to determine the site of greatest heart rate deceleration. This site is thought to index a frontal-vagal autonomic pathway that intersects a key pathway believed to underlie rTMS response.
Objective: We aimed to independently replicate previous findings of high-frequency NCG-TMS and extend it to evaluate the use of low-frequency rTMS for NCG-TMS.
Methods: Twenty healthy participants (13 female; aged 38.6 ± 13.9) underwent NCG-TMS on frontal, fronto-central (active) and central (control) sites. For high-frequency NCG-TMS, three 5 s trains of 10 Hz were provided at each left hemisphere site. For low-frequency NCG-TMS, 60 s trains of 1 Hz were applied to left and right hemispheres and heart rate and heart rate variability outcome measures were analysed.
Results: For high-frequency NCG-TMS, heart rate deceleration was observed at the left frontal compared with the central site. For low-frequency NCG-TMS, accelerated heart rate was found at the right frontal compared with central sites. No other site differences were observed.
Conclusion: Opposite patterns of heart rate activity were found for high- and low-frequency NCG-TMS. The high-frequency NCG-TMS data replicate previous findings and support further investigations on the clinical utility of NCG-TMS for optimising rTMS site localisation. Further work assessing the value of low-frequency NCG-TMS for rTMS site localisation is warranted.

Introduction

Two decades of research on the efficacy of repetitive transcranial magnetic stimulation (rTMS) for treatment resistant depression has culminated in the translation of rTMS in clinical practice in multiple countries. Spurred by its FDA approval in 2008, rTMS has provided a safer and cognitively benign alternative to ECT for some treatment resistant depressed patients who have essentially no other treatment options. Response rates for rTMS are reported as between 30% [1] and 50% [2], with emerging research suggesting that this rate may be enhanced by profiling brain structure and function prior to treatment [3,4]. Indeed, optimization of rTMS protocols is necessary to maximise response rates and reduce burden of non-response on patients and services.

A common approach for improving response rates to rTMS for depression is by advancing site localisation methods. To date,

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relatively rudimentary localisation methods are used in the clinic for which the scalp location overlying the dorsolateral prefrontal cortex (DLPFC) is estimated; namely, 5 cm, 6 cm and F3 (10–20 EEG system) methods [5]. A spate of imaging studies have underscored the importance of considering differences in inter-individual brain morphology by showing that scalp measurement methods do not consistently localise the DLPFC accurately [6–8]. The clinical applicability of MRI guided neuronavigation of the DLPFC has been demonstrated by our group with better treatment outcomes reported using this method compared with the 5 cm method [8]. However, structural MRI guided neuronavigation has not been adopted into clinical practice largely due to the translational limitations (i.e. cost and practicality of imaging).

Another significant limitation of structural MRI guided neuronavigation for rTMS is that it does not target functionally connected brain areas, i.e. it is based on structural rather than functional brain activity. There is increasing support for depression as a 'network disorder' that is characterised by altered neuronal activity within spatially and temporally distinct functional networks [4]. Specific and connectivity-related abnormalities of the DLPFC and subgenual anterior cingulate cortex (sgACC), respective nodes of the central executive network and the default mode network, are the most reproducible findings in depression [4,9]. Importantly, normalisation of DLPFC activity corresponds with antidepressant medication response [10] and, normalisation of sgACC activity corresponds with response to multiple depression treatments (e.g. SSRIs, rTMS, ECT, deep brain stimulation, vagus nerve stimulation) [9]. Moreover, rTMS reportedly normalises depression related sgACC hyperconnectivity in the default mode network and modulates the interplay between the default mode network and the central executive network (both strongly implicated in the pathology of depression) [11]. These findings support the hypothesis that rTMS exerts its therapeutic benefit through the propogation of effects from stimulation of a critical cortical node (i.e. the DLPFC) to deeper subcortical structures in one or more interconnected networks. Compelling evidence for the relationship between DLPFC to sgACC functional connectivity and rTMS treatment response is now emerging. Specifically, Fox and colleagues [4] showed in a retrospective analysis that the degree of negative (i.e. anti-) correlation of the DLPFC site of stimulation and sgACC functional connectivity predicted response to rTMS for depression, accounting for more than 70% of the variance in efficacy. The utility of DLPFC to sgACC functional connectivity in predicting clinical efficacy was further demonstrated by a prospective validation study [12] and an independent validation study of these findings [3]. Critically, this body of work provides a promising avenue for the development of functionally meaningful site localisation methods for rTMS treatment of depression that are likely to result in improvements in treatment efficacy.

A potential avenue for identifying a functionally meaningful site based on the connectivity of the DLPFC to sgACC pathway for rTMS treatment may be to probe the brain-heart connection. The brain-heart connection in the context of affective regulation has been long recognised by a plethora of neuroimaging and pharmacological studies in humans and animals [13,14]. The top-down modulation of heart rate by the prefrontal cortex involves a frontal-vagal pathway from frontal nodes (including the prefrontal cortex, anterior cingulate and insula) to subcortical nodes within the medial visceromotor network [14]. Through vagal (i.e. parasympathetic) activation, indexed by heart rate deceleration, the prefrontal cortex is touted to have a parasympathoinhibitory influence over subcortical nodes [14]. The well-known autonomic nervous system characteristics of depression (i.e. reduced parasympathetic to sympathetic balance and the high incidence of cardiovascular disease) is therefore thought to result from reduced prefrontal cortex parasympathoinhibition [15]. Within the medial visceromotor network, several lines of evidence support the sgACC as the cardinal frontal region for autonomic regulation; it directly and monosynaptically connects frontal nodes to subcortical structures (including the nucleus tractus solitarious of the vagus nerve) [9]. A meta-analysis has reported that rTMS induces a reduction in heart rate and an increase in heart rate variability of moderate effect, particularly when applied to the prefrontal cortex compared with the motor cortex. These findings suggest that rTMS applied to the prefrontal cortex results in stimulation of the frontal vagal network involved in cardiovascular control [13]. The effect of stimulating the frontal vagal network with rTMS on heart rate thus provides an opportunity for probing DLPFC-sgACC connectivity shown to underlie rTMS clinical efficacy. Neuro-cardiac-guided TMS (NCG-TMS) is a newly developed method that proposedly probes the frontal vagal network in order to individualise DLPFC site of stimulation selection for rTMS treatment [16].

Iseger and colleagues [16] published the first insight into NCG-TMS; in this pilot study ($n = 10$), the DLPFC site of greatest heart rate deceleration with rTMS was proposed as the optimal site for rTMS treatment and was shown to vary across individuals. In this study, high-frequency rTMS was applied to left and right 10–20 EEG frontal sites, F3/F4 and FC3/FC4 and, central sites C3/C4 as the control sites. For both left and right hemispheres, there was a general pattern of heart rate deceleration at frontal sites and heart rate acceleration at central cites. On the left hemisphere, the greatest heart rate deceleration was observed at F3 for 80% of participants, however, in 20% of participants the greatest heart rate deceleration was found at FC3. Similarly, on the right hemisphere, the greatest heart rate deceleration was shown at F4 for 60% of participants and at FC4 for 40% of participants. These data therefore indicate that for some participants, FC3 (and FC4) were more functionally connected to the frontal vagal network than F3 (and F4). As such, for these participants, rTMS provided at these sites may result in better treatment response compared with F3 (and F4). If these findings are replicated, NCG-TMS may be investigated as a possible strategy for personalised targeting of rTMS treatment.

The current study aimed to replicate the findings of Iseger et al. [16] on high-frequency NCG-TMS applied to the left hemisphere in a larger and independent sample. Secondarily, we aimed to evaluate the applicability of NCG-TMS for low-frequency rTMS treatment, since it is equally efficacious for depression, is better tolerated [17] and potentially safer [18,19] than high-frequency rTMS. In addition, a low-frequency NCG-TMS protocol allows for a longer period of concurrent heart rate recording during rTMS, therefore allowing us to further assess the value of heart-rate variability measures for NCG-TMS as well as testing the direct effects of this rTMS protocol.

Materials and methods

Participants and procedure

Twenty healthy participants (13 females) aged between 18 and 65 years (range = 20–62; mean = 38.6 ± 13.9) were recruited via flyers, public notice boards and on social media. Exclusion criteria were contraindications to TMS (including but not limited to the presence of metal inside the head excluding dental work, professional drivers, pregnancy or currently breast-feeding and history of seizure), history of neurological or psychiatric disorders, and history of or current cardiac abnormalities. Participants were screened for psychopathology using the M.I.N.I International Neuropsychiatric Interview [20]. Prior to testing, participants were asked to refrain from consumption of nicotine (for at least 3 h), caffeine (for at least 2 h) and alcohol (for at least 12 h), respectively and

abstinence was confirmed with participants immediately before the testing session.

After the provision of informed consent, participants underwent clinical interview to screen for psychopathology and substance use with a trained researcher (JM) and then, resting motor threshold (RMT) assessment of the left- and right-motor cortices. Thereafter, electrocardiogram (ECG) electrodes were attached and participants were seated, asked to relax, limit any movement and avoid talking during testing. Once ECG trace quality was checked via visual inspection and participants were settled, 2 min of resting ECG was recorded. High-frequency and low-frequency rTMS protocols were then administered and ECG was recorded concurrently. The order of frequency type was counter-balanced across participants.

This study was approved by the Alfred Hospital and Monash University Human Research Ethics Committees and written informed consent was provided by all participants, in keeping with the declaration of Helsinki.

Power analysis

A power analysis was conducted based on a 2-tailed *t*-test using two dependent means, with alpha set at 0.05 and power set at 90% (GPower 3.1). The calculation was based on the Cohen's *d* effect sizes achieved by the previously published pilot study (n = 10) on high-frequency NCG-TMS [16]. For the effect size of 1.0 for the comparison between F3–C3, the required sample was n = 13 and, for the effect size of 0.88 for the comparison between FC3–C3, the required sample was n = 16. Therefore, based on the available data, the sample of n = 18 in this replication study was sufficient to detect differences in heart rate between frontal and central sites with high-frequency NCG-TMS.

Neuro-cardiac-guided TMS data acquisition

Stimulation was applied using a Medtronic MagPro stimulator and a 70-mm diameter figure-of-8 coil. Single-pulse TMS was applied to the left- and right-motor cortices to measure the RMT for each hemisphere using electromyography using standard published methods [21]. ECG electrodes were attached to the middle of the breast bone (ground electrode), to the right above the right breast and to the left below the left breast. ECG was recorded during stimulation with the nCG-ENGAGE HR (neuroCare, Munich, Germany) and NCG-TMS purposely designed 10–20 EEG caps (without electrodes) were used to guide site of stimulation. Fz was the first site stimulated with each rTMS protocol in order to accustom the participant to the sensation of each type of stimulation and to titrate the intensity up to 100% left or right RMT, which ever was higher. For the high-frequency protocol, three 5 s trains of 10 Hz at 100% of RMT with a 30 s inter-train interval were applied to 3 locations (excluding Fz) on the left hemisphere, F3, FC3 and C3. Left and right central sites (C3 and C4) were stimulated as control sites for comparison to left and right frontal and fronto-central sites (F3, FC3 and F4, FC4, respectively). For the low-frequency protocol, a single 60 s train of 1 Hz at 100% of RMT with a 30 s inter-train interval was applied at 6 locations (excluding Fz) on the left (F3, FC3 and C3) and right (F4, FC4 and C4) hemispheres.

Data processing

Automatic R-peak detection was performed by the nCG-ENGAGE HR. All data were visually inspected to determine correct detection of R peaks and movement artefacts. All data were sampled at a rate of 1000 Hz. The high-frequency data analyses were identical to the study by Iseger et al. [16]. To control for the effect of respiration on heart rate, R-R values from the trough at pre-stimulation, trough 1, trough 2 and trough 3 (at each site) were used in analyses. Data were averaged and normalised to z-scores to reduce inter-individual and inter-train effects using the formula: trial 1 − trial 0/sd trial 0 (repeated for trials 2 and 3). Note, due to the short duration of high-frequency rTMS trains (i.e. 5 secs), heart rate variability measures could not be determined. The low-frequency NCG-TMS data was imported into Kubios Premium (ver. 3.0.2) [22] for analysis of mean R-R interval and heart rate variability data. Data were corrected for ectopic or misplaced beats and movement artefact by interpolation (using the automatic correction feature in Kubios Premium). Data requiring corrections for more than 5% of total R-peaks were excluded. Two heart rate variability measures known to reflect parasympathetic system activity [23] were chosen for analysis; i) the time domain measure, Root Mean Square of the Successive Differences (RMSSD) calculated from the R-R intervals, and ii) the frequency domain measure, high-frequency power log (HF-HRV; 0.15 Hz–0.4 Hz). HF-HRV was calculated by Fast Fourier Transform based on Welch's periodogram (window width of 300 s, 50% overlap).

Statistical analyses

For the high-frequency data, SPSS 23.0 (SPSS Inc., Chicago, Illinois, USA) for Windows was used to perform analyses. To replicate the differences in heart rate change between sites described by Iseger and colleagues [16], the high-frequency data were analysed using paired samples t-tests (one-tailed). Cohen's *d* effect sizes were generated to assess pair-wise differences in heart rate change between sites with high-frequency rTMS. The optimal location for individuals was determined by the site of greatest heart rate deceleration.

Analyses for the low-frequency NCG-TMS data were run with SAS 9.4 (SAS institute, Cary NC). Three (site) x 2 (hemisphere) repeated measures analyses of variance (ANOVA) were written to test the hypothesis of site differences for outcomes mean R-R interval, mean RMSSD and mean HF-HRV across left and right hemispheres. The site (F, FC, and C) and hemisphere (left, right) were modelled as within subject variables so that each participant was represented by 6 data points or rows. To minimise the correlation between the mean and variance for each outcome measure, natural log transformations were applied. Further, whenever the sphericity assumption was not met, Huynh-Feldt corrected *p* values were reported. Bonferroni post-hoc pairwise comparisons between sites within site by hemisphere (site*hemisphere) were adjusted for multiplicity using Bonferroni correction. Additionally, the effect sizes or Cohen's *d* were calculated for significant score differences within site*hemisphere. The alpha level was set at two-tailed 0.05.

Results

Participant sample

Following inspection of ECG data (see Fig. 1), eighteen participants (11 females; aged 38.7 ± 13.5) with a mean left RMT of 49.7 ± 5.9 were included in the high-frequency NCG-TMS analysis after n = 2 were excluded due to technical recording issues. No high-frequency data were lost to incorrect R peak detection. For the low-frequency data, 19 participants (12 females; aged 38.9 ± 14.1) were included in the analysis after n = 1 were excluded due to >%5 of R peaks rejected in at least one variable. For this sample, the mean RMT for left and right motor cortices were 50.0 ± 5.7 and 49.9 ± 8.9, respectively.

Fig. 1. An example of high-frequency NCG-TMS (top) and low-frequency NCG-TMS (bottom) data with the ECG trace represented in blue, the TMS triggers represented in red and the R-peak detection by the NCG-TMS module in yellow. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

High-frequency NCG-TMS

As expected, the difference in heart rate (mean z-scores) with high-frequency rTMS between F3 (mean = 1.50 ± 3.68) and C3 (mean = −0.36 ± 1.11) was significant and of medium to large effect [$t\,(17) = 2.08$, $p = .02$, $d = 0.69$]. While the difference in heart rate change between FC3 (mean = 0.22 ± 1.60) and C3 was not significant [$t\,(17) = 1.27$, $p = .22$], significance emerged for the difference between F3 and FC3 [$t\,(17) = 1.77$, $p < .05$]. Small to medium effect sizes were found for the pair-wise comparisons between FC3 and C3 ($d = 0.42$) and, F3 and FC3 ($d = 0.45$). The pattern of heart rate change across sites is displayed in Fig. 2 and percentage distributions of optimal site at the individual level are shown in Table 1.

Low-frequency NCG-TMS

The raw means and standard deviations for R-R interval, RMSSD and HF-HRV are shown in Table 2. For mean R-R interval, the analyses showed a significant effect of site [$F\,(2, 36) = 4.87$, $p = .01$]. Post-hoc comparisons revealed a significant mean difference between F4 and C4 ($d = -0.67$, p = .02). The hemisphere and site*hemisphere effects were not statistically significant at 0.05 level (see Table 3). We noted a significant hemisphere effect on RMSSD scores [$F\,(1, 18) = 5.28$, $p = .03$)] but no effect of site ($p > .05$). For HF-HRV, there were no significant effects of site, hemisphere or their interaction on scores (all $p > .05$). As noted in Table 4, no post-hoc mean differences within site within site*hemisphere have approached significance for HF-HRV or RMSSD outcomes. Simply stated, for R-R interval, while there was no effect of laterality across sites, there was an effect of site across both the hemispheres, with differences across sites showing a significant heart rate acceleration at F4 compared to C4. For RMSSD, there was a difference in left versus right hemispheres across sites but no effect of site across hemispheres. Fig. 3 shows that the pattern of R-R interval means (log transformed) across sites is similar for both hemispheres, with accelerated heart rate at frontal compared with central sites. Fig. 4 depicts the different RMSSD (log transformed) patterns across hemispheres. The percentage distributions of site with the most accelerated heart rate at the individual level are shown in Table 5.

Exploratory post-hoc analyses

In light of previous research reporting skull to cortex distance increases with age at a greater rate for the prefrontal cortex compared to the motor cortex [24], the relationships between heart

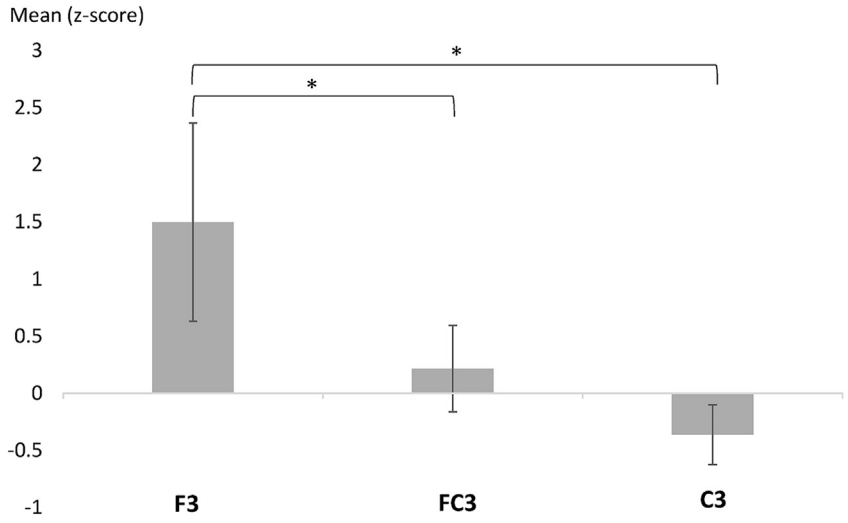

Fig. 2. A bar graph showing the pattern of heart rate change (mean z-scores and standard error) across sites of stimulation with F3 and FC3 as test sites and C3 as the control site. *Note:* * denotes significance at $p < .05$.

rate with high- and low-frequency NCG-TMS and age were explored post-hoc using Pearson's correlation analyses. For high-frequency NCG-TMS, no significant relationships between heart rate at F3, FC3 or C3 ($r = 0.01$, $r = 0.06$ and $r = -0.17$, respectively) and age were observed (all $p > .05$). Similarly for low-frequency NCG-TMS, no significant relationships between heart rate at F3, FC3 or C3 ($r = 0.07$, $r = 0.03$ and $r = 0.04$, respectively) and age were found (all $p > .05$). The means and standard deviations for age are included in Tables 1 and 5.

The overlap between optimal sites for high- and low-frequency NCG-TMS on the left hemisphere was assessed for the n = 17 participants who had useable NCG-TMS data for both high- and low-frequency protocols. For 47% of participants, the frontal site of greatest heart rate deceleration with high-frequency NCG-TMS was the same as the frontal site of greatest heart rate acceleration with low-frequency NCG-TMS, for which the probability was 50%. Similarly, the observed proportion (32%) was similar to the expected proportion (33.3%) of participants who had the same overall site of greatest heart rate deceleration with high-frequency NCG-TMS and overall site of greatest heart rate acceleration with low-frequency NCG-TMS.

Discussion

The present study forms part of an early literature on NCG-TMS and is the first independent replication study of heart rate deceleration with high-frequency NCG-TMS at F3 compared to C3, where heart rate acceleration is observed. In addition, while not significant, a similar pattern of heart rate deceleration was seen at FC3 compared with C3. At the individual level, these data showed marked inter-individual variability in the left hemisphere site of greatest heart rate deceleration. Additionally, the current study is the first to assess low-frequency NCG-TMS for which we showed no effect of laterality but an effect of site with accelerated heart rate at F4 compared to C4. For heart rate variability measures with low-frequency NCG-TMS, there was a laterality effect for RMSSD, however, there were no post-hoc site differences within the site by hemisphere interaction. There was no effect of hemisphere, site or site by hemisphere interaction for HF-HRV. Marked inter-individual variability in site of greatest accelerated heart rate for left and right sites with low-frequency NCG-TMS were noted, with a similar pattern of distribution compared with high-frequency NCG-TMS. Further inspection of the data revealed that the proportion of overlap in greatest site of heart rate deceleration and acceleration with high- and low-frequency NCG-TMS, respectively, was consistent with the proportion expected by chance. These high-frequency NCG-TMS findings replicate the earlier pilot study [16] supporting that the frontal vagal pathway may be probed using high-frequency NCG-TMS, with the frontal location of vagal activation varying across individuals. Furthermore, this study suggests that the low-frequency rTMS may have utility for NCG-TMS and highlights the differential physiological effects of low- and high-frequency rTMS.

Our independent replication of frontal patterns of heart rate deceleration with high-frequency rTMS corroborates previous NCG-TMS findings [16] and the meta-analysis of the effect of rTMS on heart rate [13]. These data support the theory that rTMS of the prefrontal cortex engages the frontal vagal pathway [14] which critically intersects the DLPFC to sgACC pathway thought to underpin the rTMS therapeutic mechanism [3,4,12]. On inspection of the optimal site distribution at the individual level, for the majority of participants, the greatest heart rate deceleration was at F3 followed by FC3, in keeping with the NCG-TMS [16] and the broader rTMS site localisation literature [25]. However, it was unexpected that in a small number of participants (n = 3), greatest heart rate deceleration was at C3, the control site. This could be attributed to individual variability in site of greatest connectivity to the sgACC or brain morphology. Another explanation relates to factors that could not be kept constant for stimulation at different sites (at different times) such as anxiety levels or discomfort during stimulation which may have influenced the sympathetic to parasympathetic balance. In this instance, it is plausible that for some individuals, stimulation on F3 or FC3 could have been more uncomfortable than at C3 and, the ensuing increased sympathetic activity could mask potential parasympathetic activity. Another point for discussion is

Table 1
A table presenting the number (N) and percentage (%) of individuals with the greatest heart rate deceleration at each site (i.e. the chosen optimal site) with high-frequency NCG-TMS. Means and standard deviations are reported for each group.

	F3	FC3	C3
N	10/18	5/18	3/18
%	55.6%	27.8%	16.7%
Mean age ± SD	42.6 ± 13.0	35.0 ± 14.3	31.7 ± 14.2

Table 2
The raw means ± standard deviations for the left (F3, FC3, C3) and right (F4, FC4, C4) sites for R-R interval, RRMSD and HF-HRV power (log) for low-frequency NCG-TMS.

	Left hemisphere			Right hemisphere		
	F3	FC3	C3	F4	FC4	C4
R-R	863.7 ± 131.8	862.4 ± 135.4	868.0 ± 130.4	850.4 ± 131.9	855.0 ± 125.9	864.3 ± 129.8
RMSSD	32.9 ± 19.6	31.3 ± 17.3	34.0 ± 19.0	30.1 ± 16.6	32.1 ± 19.6	30.0 ± 19.0
Log HF-HRV	5.7 ± 1.3	5.7 ± 1.5	5.7 ± 1.4	5.4 ± 1.5	5.7 ± 1.4	5.5 ± 1.4

Table 3
The repeated measures ANOVA results for site, hemisphere and site by hemisphere interaction with corresponding *F* statistics (degrees of freedom, error degrees of freedom) and *p* values for R-R interval, RRMSD and HF-HRV (all log transformed) for the low-frequency NCG-TMS sample (n = 18). *Note:* * denotes p < .05.

Outcome	Site		Hemisphere		Site*Hemisphere	
	F(2, 36)	*p*	*F*(1, 18)	*p*	*F*(2, 36)	*p*
Log R-R	4.87	0.01*	3.90	0.06	1.20	0.31
Log RMSSD	0.09	0.91	5.28	0.03*	2.56	0.09
Log HF-HRV	0.68	0.51	3.97	0.06	2.60	0.09

Table 4
Mean differences matrix for Bonferroni pairwise comparisons for each outcome (log transformed) for site within site*hemisphere. *Note*: * denotes *p* < .05.

Outcome	Hemisphere	Site	Mean	F	FC
Log R-R	Left	F	6.7479	–	–
		FC	6.7461	0.0018	–
		C	6.7537	0.0057	0.0075
	Right	F	6.7324	–	–
		FC	6.7388	0.0064	–
		C	6.7497	0.0173*	0.0109
Log HF-HRV	Left	F	5.7199	–	–
		FC	5.6645	0.0554	–
		C	5.7389	0.0191	0.0745
	Right	F	5.4049	–	–
		FC	5.6906	0.2857	–
		C	5.4626	0.0577	0.2280
Log RMSSD	Left	F	3.3191	–	–
		FC	3.2796	0.0395	–
		C	3.3625	0.0434	0.0829
	Right	F	3.2412	–	–
		FC	3.2666	0.0254	–
		C	3.2093	0.0319	0.0573

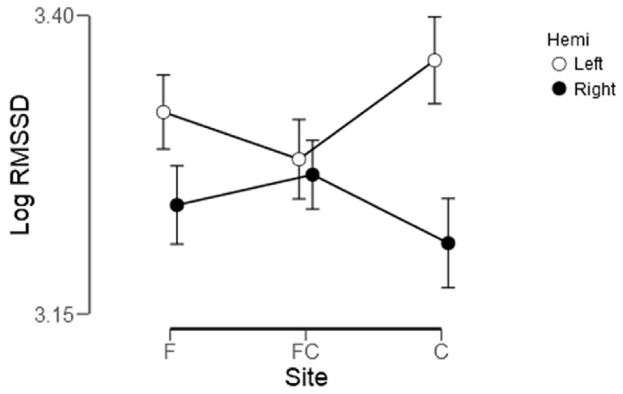

Fig. 4. A line graph depicting the pattern of root mean square of the successive differences (log transformed) across the frontal (F), fronto-central (FC) and central (C) sites for left (white) and right (black) hemispheres for low-frequency NCG-TMS.

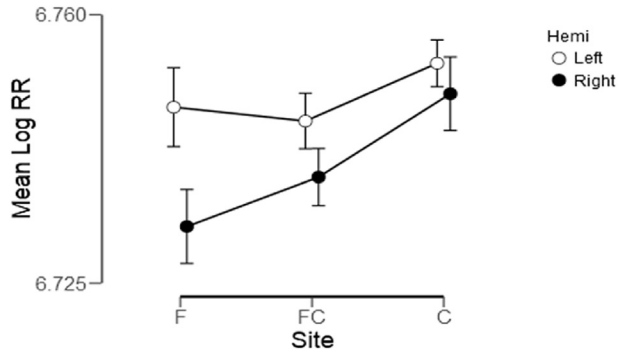

Fig. 3. A line graph depicting the pattern of R-R interval means (log transformed) across the frontal (F), fronto-central (FC) and central (C) sites for left (white) and right (black) hemispheres for low-frequency NCG-TMS.

that heart rate deceleration at FC3 compared to C3 was not significant, unlike the previous study [16]. Notwithstanding, the effect size for this comparison (d = 0.42) is similar to the effect size for FC3/FC4 compared with C3/C4 (d = 0.47) generated by a recent individual participant meta-analyses [26]. The meta-analysis combined available high-frequency NCG-TMS datasets, including the data in the current study (n = 66 healthy participants with left and/or right hemisphere data), with the objective of increasing power and to test any laterality effects. Importantly, the meta-analysis substantiates the findings in this study by showing heart rate deceleration at F3/F4 compared to C3/C4 (d = 0.56) and at a lesser degree, FC3/FC4 compared to C3/C4. Further, there was no significant effect of laterality.

The difference in heart rate with low-frequency NCG-TMS between F4 and C4 is consistent with high-frequency NCG-TMS findings [16] despite that heart rate is accelerated at F4 compared with C4 (rather than decelerated as observed with high-frequency NCG-TMS) which, taken together with the null findings in heart rate variability measures of parasympathetic activity, reflects net sympathetic over parasympathetic activity [14]. The difference between F4 and C4 is moderate to large in effect and the visual heart rate pattern across sites on both hemispheres is similar (albeit non-significant on the left), lending some confidence in the finding. Of note, cerebral laterality effects of cortical autonomic control have previously been documented [27,28], including a study showing more pronounced effects of low-frequency rTMS on the right hemisphere [29], in line with the current results.

Table 5
A table presenting the number (N) and percentage (%) of individuals with the greatest accelerated heart rate (R-R interval) at each site within left and right hemispheres with low-frequency NCG-TMS. Means and standard deviations are reported for each group.

	F3	FC3	C3	F4	FC4	C4
N	8/19	7/19	4/19	11/19	6/19	2/19
%	42.1%	36.8%	21.1%	58%	31.6%	10.5%
Mean age ± SD	33.9 ± 12.6	42.6 ± 15.6	42.8 ± 14.9	42.0 ± 14.1	33.8 ± 13.0	37.5 ± 21.9

The opposite pattern in heart rate measures with high and low-frequency rTMS is consistent with the broader literature reporting of differential biological effects of these rTMS types [30]. While the specific effects of low- and high-frequency rTMS are not clear, the most replicable finding for low-frequency is that it produces a decrease in cortical excitability, whereas, more contentiously, high-frequency produces an increase in cortical excitability and potentially, a reduction in cortical inhibition. It is possible that with low-frequency rTMS on the prefrontal cortex, the net effect is a dampening of parasympathoinhibition which then allows an increase in sympathoexcitation. With high-frequency, if the net effect is increased cortical excitability, this could result in parasympathoinhibitory potentiation. It is also worth noting that low-frequency trains were far longer than high-frequency trains (1 min vs 5 s); the effect of low-frequency rTMS on heart rate was measured by net heart rate change over this period and any short-term heart rate changes with rTMS may have been masked. Unexpectedly, this study did not show any greater spatial overlap between site of greatest heart rate deceleration and acceleration with high- and low-frequency rTMS, respectively, than expected by chance. This suggests that the net sympathetic activity may have been elicited through a different frontal pathway. Indeed, indirect and direct sympathoexcitatory and parasympathoinhibitory pathways are involved in the frontal modulation of the autonomic network [14]. Thus, the low-frequency protocol could have probed an alternate frontal pathway which leads to net sympathetic activity. However, these interpretations should be considered with caution given the complex intersection in understanding the effect of rTMS frequency types and the physiology of autonomic control.

To advance this emerging field of research, mechanistic neurobiological studies to validate proposed NCG-TMS mechanisms and on samples of depressed patients are required to assess its value in guiding site localisation for rTMS treatment of depression. Moreover, while we found no significant relationships between heart rate with NCG-TMS and age in this study, future research should explore age a confounding factor. In the context of rTMS, age effects appear to be particularly relevant for those over 55 years old [24] and since only 1 participant was over 55 years old in the current study, any potential effect of age in this study is likely to be minimal. Notably, through measuring an observable effect of activation of the frontal vagal pathway (i.e. heart rate), the NCG-TMS method could likely be used in future studies to establish a true frontal threshold, similar to the motor threshold, and thus resolve the possible issue of differences in frontal and motor cortices. It is also unclear to what extent the sensory experience of rTMS on different frontal and central 10–20 sites influences heart rate, a potential limitation that deserves evaluation in future research. Further, the low-frequency NCG TMS data could not be corrected for baseline heart rate (unlike for high-frequency NCG-TMS); due to the dynamic nature of heart rate fluctuations over the 1 min length of the heart rate recording, any baseline correction obscured the results. Therefore, heart rate at each site for this condition was compared relative to heart rate at the other sites. Lastly, although a 'practice' site (i.e. Fz) was included in this protocol to accustom participants to the sensation and to up titrate the intensity of rTMS, the stimulation order of sites was not controlled for in this study and any site order effects may have influenced the results.

Conclusions

The present study extends previous findings on high-frequency NCG-TMS and provides further evidence that rTMS applied on frontal sites can modulate autonomic control via a frontal vagal pathway which is thought to overlap the DLPFC-sgACC pathway underlying rTMS efficacy for depression. If the proposed mechanism of NCG-TMS is validated, high-frequency NCG-TMS may have potential as an affordable method for individualised targeting of site for rTMS treatment of depression. While further research is needed to assess the value of low-frequency rTMS for NCG-TMS, these data substantiate the extant literature showing differential effects of high- and low-frequency rTMS.

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Contributors

MK, KH, BMF, MA and PBF were involved the study design and writing of the protocol; MK and JAM performed the literature searches; JAM and MSR recruited and performed experimental testing of subjects; MK, JAM, MSR and TI developed and performed raw data analysis; MK, KH, BMF and PBF managed the study; MK and ARH conducted statistical analyses. All authors contributed to the interpretation of the data and have approved the manuscript.

Declaration of competing interest

PBF has received equipment for research from MagVenture A/S, Medtronic Ltd, Neuronetics and Brainsway Ltd and funding for research from Neuronetics. He is on scientific advisory boards for Bionomics Ltd and LivaNova and is a founder of TMS Clinics Australia. MA reports options from Brain Resource (Sydney, Australia), is unpaid research director of the Brainclinics Foundation, a minority shareholder in neuroCare group (Munich, Germany); TAI and MA are co-inventor on a patent application covering NCG-TMS, but do not own the patent nor receive any royalties related to this patent; Research Institute Brainclinics received research funding from Brain Resource (Sydney, Australia) and neuroCare group (Munich, Germany); equipment support from Deymed, neuroConn and MagVenture, however data analyses and writing of this manuscript were unconstrained. We have no other conflicts of interest to declare.

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