scientific reports

Check for updates

OPEN

Continuous theta burst stimulation at 30 hz does not modulate cortical excitability in a sham-controlled study

Joana Frieske[1,2]✉, Sybren Van Hoornweder[1], Marten Nuyts[1], Stefanie Verstraelen[1], Stephan P. Swinnen[2] & Raf L.J. Meesen[1,2]

Theta burst stimulation (TBS) can modulate cortical excitability but suffers from high inter-subject variability. Modified TBS frequency patterns (30 Hz) showed consistent inhibitory aftereffects, but further research into the time course of these effects is needed. This study aimed to investigate the efficacy of a 30 Hz continuous TBS (cTBS) protocol. Participants ($n = 20$) underwent an experimental session (real cTBS) and a control session (sham cTBS). To assess cortical excitability, Transcranial Magnetic Stimulation was applied over the primary motor cortex before cTBS, and at five timepoints after cTBS. Percentage change (PC) to baseline was analysed using a Linear Mixed Model. No difference in PC was found between real and sham cTBS ($p = 0.696$). Our results demonstrate a significant increase in PC over time ($p = 0.006$) at 30, ($p = 0.01$), 45 ($p = 0.027$), and 55 min ($p = 0.024$) post cTBS, irrespective of condition. Secondary analysis dividing the sample into responders and paradox-responders showed no significant predictors for cTBS responsiveness. We could not replicate previously reported suppressive effects of 30 Hz cTBS. Increases in MEP amplitudes over a 60-minute time window were independent of stimulation condition and marked by high inter-subject variability. Validations of modified TBS protocols are further needed to replicate findings and understand mechanisms underlying individuals' responsiveness.

Keywords Transcranial magnetic stimulation (TMS), Continuous theta burst stimulation (cTBS), Variability, 30 hz cTBS, Responsiveness, Cortical excitability

Transcranial magnetic stimulation (TMS) is a non-invasive brain stimulation technique that can be used to assess cortical excitability and modulate neuronal activation patterns[1]. While the former is typically achieved via single TMS pulses, the latter can be attained via repetitive TMS (rTMS), which generates multiple high frequency pulses leading to transient post-stimulation changes in neuroplasticity[1,2].

Theta burst stimulation (TBS) is an rTMS protocol that was shown to demonstrate benefits over conventional rTMS protocols as it achieved neuroplastic changes in a shorter timeframe, by applying fewer number of pulses with a lower intensity[3]. Depending on the temporal sequencing, TBS was initially found to either enhance or decrease cortical excitability, which was defined as intermittent TBS (iTBS) or continuous TBS (cTBS) respectively[4,5].

The cTBS protocol of Huang et al. (2005), which has become the standard protocol in use, induces triplets of pulses with a frequency of 50 Hz. These triplets are repeated every 200 ms (5 Hz) over a time frame of 20–40 s. Applying a total of 600 pulses over 40 s has been shown to induce a suppression of cortical excitability up until 60 min following cTBS[5]. However, the time course of the effect varies noticeably across different studies. A meta-analysis by Chung et al. (2016) found significant reductions of cortical excitability up until 60 min following cTBS, with the strongest effect being present at 5 min[2]. Others have found suppressive effects peaking 10–20 min post intervention[6]. Contrasting results were found by a large-scale analysis which reported a significant reduction of motor evoked potentials (MEP) up until only 10 min post cTBS[7]. Additionally, there seems to be an

[1]REVAL - Rehabilitation Research Center, Faculty of Rehabilitation Sciences, University of Hasselt - Campus Diepenbeek, Wetenschapspark 7, Diepenbeek B-3590, Belgium. [2]Movement Control and Neuroplasticity Research Group, Department of Movement Sciences, Group Biomedical Sciences, KU Leuven, Leuven, Belgium. ✉email: joana.frieske@uhasselt.be

overall lack of adequate sham-controlled studies in rTMS and TBS research[8], which undermines the validity of conclusions drawn from previously observed significant effects.

With the implementation of TBS protocols becoming more and more widespread, it has become evident that subsequent cortical excitability changes are notoriously variable, both across- and within-subjects[9,10]. This observation has sparked previous researchers to investigate which factors may explain across- and within-subject differences. For instance, Corp et al., 2020 found that methodological factors such as baseline MEP peak-to-peak amplitude, target muscle, age and time of day contributed to the observed high inter-individual variabilities[7]. Furthermore, differences in the recruitment of I-waves, brain-derived neurotrophic factor (BDNF) genotypes, age, sex, and methodological differences have also been put forward as potential causes of TBS response variability[11–15]. To identify which persons do respond to TBS, research dividing the sample into responders vs. non-responders based on contrasting effects following stimulation has previously been conducted[13]. However, a lack of knowledge about the underlying mechanisms characterizing responders calls for more investigation into individual-specific factors.

Akin to most non-invasive brain stimulation modalities, TBS is characterized by an extensive parameter space. Previous work has highlighted that isolated deviations from the standard protocol can already lead to vastly different TBS after-effects. For instance, in their search for an optimal number of pulses, McCalley et al., 2021 showed that doubling the number of pulses from 600 to 1200 reversed the effect of cTBS on cortical excitability from inhibition to facilitation[16]. A similar effect was shown when increasing the stimulation intensity to 100% of active motor threshold (aMT) instead of the commonly used 80% aMT[17]. Measuring cortical excitability via single-pulse TMS on M1 could be an additional factor amplifying the high variability observed in previous studies. Research investigating the effects on variability comparing multiple stimulation intensities via input-output curves suggested that MEP amplitudes might be less variable on the upper end of the curve[18]. 150% of resting motor threshold (rMT) was found to be the most reliable stimulation intensity to capture cTBS suppressive effects due to the near-maximal late I-wave recruitment. The recruitment of near-maximum late I-waves was implied to lower variability and increase detection of early I-wave suppression induced by cTBS[18,19]. Since mechanisms underlying these effects are not well understood, more studies are needed to disentangle how certain protocol adaptations affect underlying neuronal mechanisms and cortical excitability.

A study by Goldsworthy et al., 2012 revealed promising results with respect to stabilizing TBS after-effects and lowering inter-subject variability. They examined alterations to the frequency of pulses which are typically applied at 50 Hz, using a frequency of 30 Hz instead. Their protocol entailed 600 pulses applied in triplets every 167ms (i.e., ~6 Hz), whereby the three pulses within one train were applied at 30 Hz instead of the common 50 Hz. By comparing the standard 50 Hz cTBS with the modified 30 Hz cTBS, they demonstrated that 30 Hz cTBS induced a more consistent and stronger suppression of cortical excitability over a time course of 30 min[20]. Despite their promising results, only a few studies have adopted the 30 Hz cTBS protocol to probe its effects on cortical excitability, with all these studies supporting the stable suppression of MEP amplitudes[21,22]. However, sample sizes ranged from 9 to maximally 12 participants and none of these studies have tested cortical excitability effects in time windows exceeding 30 min in a sham-controlled design. Accordingly, whether a modified cTBS protocol might overcome the highly variable effects of TBS over a 60-minute time course is yet to be investigated. Certainly, it is important to extend research on modified experimental paradigms and the minimization of inter-subject variability using adequate sham conditions to enhance the reliability of TBS.

The aim of this study was to investigate the variability and duration of cTBS aftereffects on cortical excitability using a modified 30 Hz protocol. We explored the length and consistency of its aftereffect by comparing 30 Hz cTBS with a sham control condition over five different timepoints (0, 15, 30, 45, 55 min). We hypothesized to observe a decrease in cortical excitability in the experimental cTBS condition by means of reduced peak-to-peak MEP amplitudes in all post-stimulation timepoints compared to baseline. Also, given that previous research showed 30 Hz cTBS to result in robust MEP suppression, we expected to see a consistent effect of MEP suppression across participants throughout the experiment.

Methods

Participants

A sample of 20 healthy volunteers was included in the study (aged 19–28, mean age ± SD: 22.35 ± 2.21 years, 11 female). Prior to inclusion, participants filled in the Edinburgh Handedness Inventory[23] and a TMS safety screening questionnaire to ensure eligibility. Participants were excluded if they showed any history of neurological and/or psychiatric disease, were regular smokers, used chronic medication or illicit drugs affecting the central nervous system, and/or had contraindications for TMS. All participants were right-handed and provided full written informed consent before participation. All methods were carried out in accordance with Declaration of Helsinki. The study was approved by the local Ethics Committee of UZ/KU Leuven (reference S66028).

Experimental design and procedure

This sham-controlled, single blinded study consisted of two sessions, which were separated by a week. All participants underwent an experimental session and a control session, of which the order was randomised. Both sessions were identical except for the stimulation condition (real versus sham cTBS) and took place at the same time of day for each participant. Participants were seated comfortably in a chair with both arms resting on a table and a monitor in front of them. During the experiment, participants were presented a digital slide show of nature images to keep their state of attention constant. Surface Ag-electrodes (Bagnoli™ DE-2.1 EMG Sensors, DELSYS Inc, Boston, MA, USA) with single-use double-sided adhesive skin interfaces (DELSYS Inc, Boston, MA, USA) were placed over the right first dorsal interosseous (FDI) and the reference electrode on the bony part of the dorsal wrist. Raw electromyographic (EMG) signals were measured, (Bagnoli-4 EMG System, DELSYS Inc, Boston, MA, USA) and 50 Hz line noise was eliminated (HumBug, Quest Scientific, North Vancouver, BC,

Canada). Following amplification (gain = 1000) and bandpass filtering (20–2000 Hz), signals were digitised at 5 k Hz (CED 1401 micro, CED Limited, Cambridge, UK) and stored on a computer for offline analysis.

The hotspot of the FDI was mapped using a 1 cm-spaced rectangular 19 × 19 cm target grid in the Brainsight® software by placing the coil over the left primary motor cortex and inducing the strongest and most consistent motor evoked potentials (MEPs) averaged over 5 consecutive TMS pulses[24]. The resting motor threshold (rMT) was determined as the lowest intensity evoking at least 5 out of 10 MEPs with a peak-to-peak amplitude larger than 50 μV on the FDI hotspot. Depending on the stimulation condition, either real cTBS or sham cTBS was applied. In both sessions, single-pulse TMS over M1 with an intensity of 150% rMT was followed immediately after, as well as 15, 30, 45, and 55 min following cTBS in blocks of 25 consecutive pulses (inter-pulse interval: 10 s +/- 20%; duration: ~4 min, 0.08 –0.11 Hz) to assess cortical excitability. Two blocks of 25 single pulses were applied prior to cTBS for baseline reference (Fig. 1). Corticospinal excitability assessment was performed with a MagPro X100 stimulator (MagVenture A/S, Farum, Denmark) connected to a figure-of-eight coil (Magventure, MC-B70). At the beginning and end of each session, participants filled in the TMS adverse events and associated sensations questionnaire (TMSensQ)[25]. After participation, participants were asked to indicate whether they thought they received real or sham stimulation.

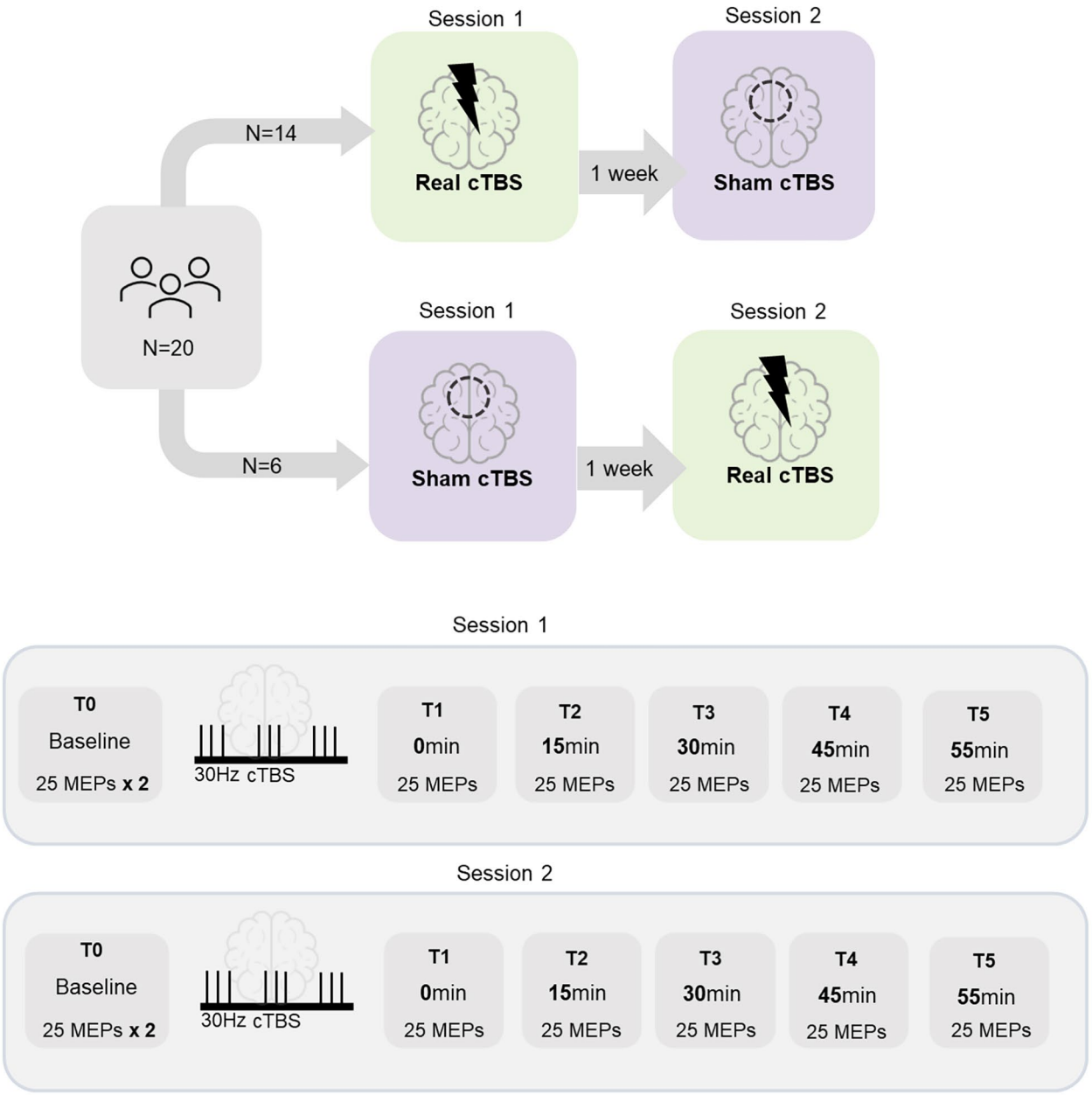

Fig. 1. Design and timepoints of experimental (real cTBS) and control condition (sham cTBS). MEPs = motor evoked potentials, cTBS = continuous theta burst stimulation.

cTBS paradigm

We used a cTBS protocol, which was modified using biphasic anterior posterior – posterior anterior (AP-PA) three-pulse bursts separated by 33.3 ms (30 Hz) induced every 167ms (~6 Hz) leading to the total stimulation duration of 33.3 s. A total of 600 pulses were given within the time frame of 33.3 s[20] with 70% rMT intensity using a 70 mm figure-of-eight coil (MCF-B70) connected to the MagPro X100 stimulator (MagVenture A/S, Farum, Denmark). In the control condition, the same protocol was applied using a sham coil (MC-P-B70). An additional 3D printed spacer (3.3 cm thickness), was attached to the sham coil to increase the distance to the scalp and further attenuate the electric field induced in the cortex[26,27].

Data processing and analysis

Data were pre-processed in MATLAB v9.13.0 (R2022b, the Mathworks, Massachusetts). Statistical analyses were performed in R (Version 4.2.2) and RStudio using packages 'lme4', 'car', 'performance', 'sjstats'[28–31]. Per trial, peak-to-peak MEP amplitude was calculated as the difference between the maximum and minimum value from 20 to 100 ms following a single TMS pulse. Trials in which EMG root mean square (RMS) exceeded 100 µV within the time window of 200 ms prior to the TMS pulse were excluded from analysis[32,33]. Single-pulse peak-to-peak MEP amplitudes were averaged over trials for each timepoint. Mean MEP amplitudes were normalised to baseline (mean of baseline block 1 and 2) and expressed as percentage change (PC) from baseline for each post-cTBS timepoint $\frac{(timepoint_x - mean\ baseline)}{mean\ baseline} \times 100$. The difference in percentage change (ΔPC) between conditions was calculated by subtracting sham cTBS PC from real cTBS PC for each post-cTBS timepoint. Based on the grand average ΔPC of all timepoints participants were divided into responders (ΔPC < 0) and paradox-responders (ΔPC > 0). In addition, we extracted MEP onset latencies. After visual inspection, we defined MEP onset automatically based on EMG signals exceeding 3 times the standard deviation from the pre-stimulus average signal. Baseline-latency was defined as the average of the two baseline blocks.

Statistical analysis

A linear Mixed Model was constructed with PC as dependent variable, and condition (real vs. sham), timepoint (T1, T2, T3, T4 and T5) and their interaction as fixed factors and participant as a random intercept. Statistical significance was set at $p < 0.05$. Shapiro-Wilk tests were conducted to test for normality of residuals and multiple comparisons were Bonferroni corrected. Binomial logistic regression was implemented using responders (responder vs. paradox-responder) as the dependent variable and age, sex, rMT, time of day (morning vs. midday vs. evening) and baseline-latency as independent factors to predict which factors might impact cTBS responsiveness. In all models, backwards model building was performed, removing the fixed factors with the highest p-values not included in any higher-level interactions first.

Results

Participant's demographics and baseline neurophysiological measures are shown in Table 1.

No effect of cTBS on cortical excitability

Average PC values and their standard deviations are visualised in Fig. 2 accompanied by individual data points of each participant.

In a first analysis, we aimed to assess whether 30 Hz cTBS is effective at modulating cortical excitability at 5 timepoints ranging from immediately after cTBS to 55 min post-cTBS.

There was no significant difference between real and sham cTBS as linear mixed model analysis revealed no significant effect of condition ($F_{1,171} = 0.99$, $p = 0.696$) and no significant condition*timepoint interaction ($F_{4,171} = 0.54$, $p = 0.706$). However, a significant effect of timepoint remained in the model after backwards building ($F_{4,176} = 3.77$, $p = 0.006$). Bonferroni-corrected multiple comparisons showed significant differences between T1 and T3 ($p = 0.01$), T1 and T4 ($p = 0.027$) and T1 and T5 ($p = 0.024$), indicating that corticospinal excitability increased over time in both the real and sham conditions.

Across both real and sham cTBS, PC values at all timepoints were consistently positive, emphasizing the absence of MEP amplitude suppression post-cTBS timepoints, compared to baseline.

No significant predictors of cTBS responders vs. paradox-responders

Beyond examining the impact of cTBS on cortical excitability on the group-level, we set out to investigate if certain participant-related variables could explain their response to cTBS. Based on the grand mean across all timepoints (Fig. 3B), participants were divided into groups of responders (ΔPC < 0) and paradox-responders (ΔPC > 0). Mean values of ΔPC of each group per timepoint are visualised in Fig. 3A.

Following backward model building, no significant fixed effects remained in the binomial logistic regression model ($\chi^2_3 = 1.7$, $p = 0.63$). Thus, our results indicate that neither sex ($\beta_{female} = 1.32$, $z = 0.775$, $p = 0.438$), age ($\beta = -0.206$, $z = -0.734$, $p = 0.463$), rMT ($\beta = 0.127$, $z = 1.088$, $p = 0.276$), time of day ($\beta = -1.495$, $z = -1.612$, $p = 0.107$) nor baseline-latency ($\beta = 52.854$, $z = 0.083$, $p = 0.934$) were successful at predicting who responds to cTBS.

Discussion

The aim of this study was twofold. First, we investigated the efficacy of a modified 30 Hz cTBS protocol, which was previously found to induce more persistent suppression of MEP amplitudes than standard 50 Hz cTBS protocols lasting up to 30 min[20–22]. Second, we extended the time window of investigating cTBS aftereffects to 60 min. We were unable to replicate previous results. Our results revealed high inter-subject variability on the immediate and delayed after-effects of cTBS and no significant suppression of cortical excitability in this sham-controlled 30 Hz cTBS paradigm at whole group level.

Participant	Sex	Age (years)	Time of day	Caffeine units < 24 h	rMT, in %MSO	cTBS intensity, in %MSO
01	male	22	09:30	1 (0)	41.5 (1)	32 (5.66)
02	male	22	14:00	0.5 (0.71)	25 (0)	21 (1.41)
03	male	23	13:30	1 (0)	34.25 (0.5)	29.5 (0.71)
04	female	24	09:30	0 (0)	30.5 (0.58)	27.5 (2.12)
05	female	21	09:30	2 (1.41)	31.5 (0.58)	27 (1.41)
06	male	22	13:30	0 (0)	36.5 (1.29)	30.5 (2.12)
07	female	25	09:30	0 (0)	33.5 (1)	28 (0)
08	female	24	13:30	0 (0)	39.25 (0.96)	33.5 (3.54)
09	female	23	13:30	0.5 (0.7)	40.25 (2.21)	34.5 (3.54)
10	male	19	09:30	0 (0)	39.5 (2.65)	33 (1.41)
11	male	21	13:45	0 (0)	36 (1.15)	29 (0)
12	female	19	09:30	0.5 (0.7)	33.7 (2.08)	32 (2.83)
13	female	24	13:30	0 (0)	41.75 (0.96)	34.5 (0.71)
14	female	21	16:30	1.5 (0.7)	25 (0.82)	24 (2.83)
15	female	28	09:15	0 (0)	38 (1.41)	32.5 (0.71)
16	female	21	13:30	0 (0)	41.75 (0.96)	35 (1.41)
17	female	20	13:30	0 (0)	28.25 (0.5)	22.5 (0.71)
18	male	21	16:30	0 (0)	37 (2.3)	31 (1.41)
19	male	22	16:30	0 (0)	44.5 (1.91)	37.5 (0.71)
20	Male	25	13:30	0 (0)	29.75 (0.96)	26.5 (2.12)
		22.35 (2.21)		**0.35 (0.39)**	**35.37 (0.71)**	**30.05 (1.39)**

Table 1. Participant's demographics and neurophysiological measures: Mean (SD) across both sessions. rMT = Resting motor threshold; cTBS = continuous theta burst stimulation; %MSO = percentage of maximum stimulator output. Note: Last row indicates group Mean (SD).

Noticeably, there was a significant increase of MEP amplitudes over time. However, this effect was not only present in the experimental but also in the sham condition, which highlights the critical importance of including sham control conditions in TBS research. As shown in a study by Magnuson et al., 2023, single session rTMS and TBS paradigms might not induce clear neuromodulatory effects when a robust sham control condition is included in the experimental design[8]. Our study is consistent with these findings and calls for careful interpretation of previously obtained results, depending on the experimental designs being used. In addition to the overall high variability and relatively low reproducibility of results from previous studies[10,11,27], the efficacy of TBS effects on cortical excitability might be less reliable than initially thought given the lack of sham-controlled conditions.

The increase of MEP amplitude across conditions over time in this study might be due to cumulative effects of single-pulse TMS[34] and/or placebo effects or state-dependent changes as a result of the experimental environment. Longer inter-stimulus intervals (ITIs) of 10s (+-20%) were chosen to counteract cumulative effects, since ITIs of 10–15 s were found to show lower variability and high reliability across sessions[35]. Besides, circadian rhythmicity as well as caffeine consumption could have impacted state-dependency and influence cTBS responses[36]. As suggested by Ly et al. 2016, corticospinal excitability slightly decreased from afternoon to evening hours and increased during the biological night[37]. However, the majority of the sessions in our study took place in the morning / midday hours and caffeine intake within the last 24 h was kept at a minimum as reported in Table 1. Research suggested no direct impact on resting state MEP amplitudes after acute caffeine intake[38]. Nevertheless, fluctuations of alertness during one session could have contributed to increases in MEP amplitudes in our study. Previous research indicated an increase in MEP amplitudes at an initial stage of drowsiness measured via Electroencephalography (EEG) flattening[39].

Additionally, recruitment of I-waves potentially impacts neuromodulatory effects depending on stimulus intensity, direction and type of stimulation. For instance, 150% rMT with a posterior-anterior current was shown to induce near maximum recruitment of late-I waves[18,19]. Crucially, cTBS effects on cortical excitability were found to reduce amplitudes of early I-waves rather than late I-waves[40]. But these expected inhibitory effects following cTBS were associated with the efficiency of recruiting late I-waves, which varied strongly between individuals and their interneuron networks[13]. Vallence et al. 2015 found that 150% rMT was the optimal stimulus intensity reliably capturing the suppressive effects of cTBS interventions which might be due to lower variability across late I- waves and therefore improved detection of early I-wave suppression[18,41].

Furthermore, the relatively high stimulus intensity of 150% rMT has been reported to initiate D-waves, next to I-waves[40,41]. As research by Vallence et al., 2015 has suggested, 180% rMT stimulus intensities showed less reliable suppression of MEPs following cTBS potentially due to the influence of D-waves which poses difficulties in detecting early I-wave suppression[18]. The contamination of D-waves that mask the effects of cTBS on I-waves may not be restricted to 180% rMT but may be an explanation for the current negative results.

Due to the high variability in responses among participants, our secondary analysis explored factors that may explain an individual's responsiveness to cTBS by dividing the sample into responders and paradox-responders.

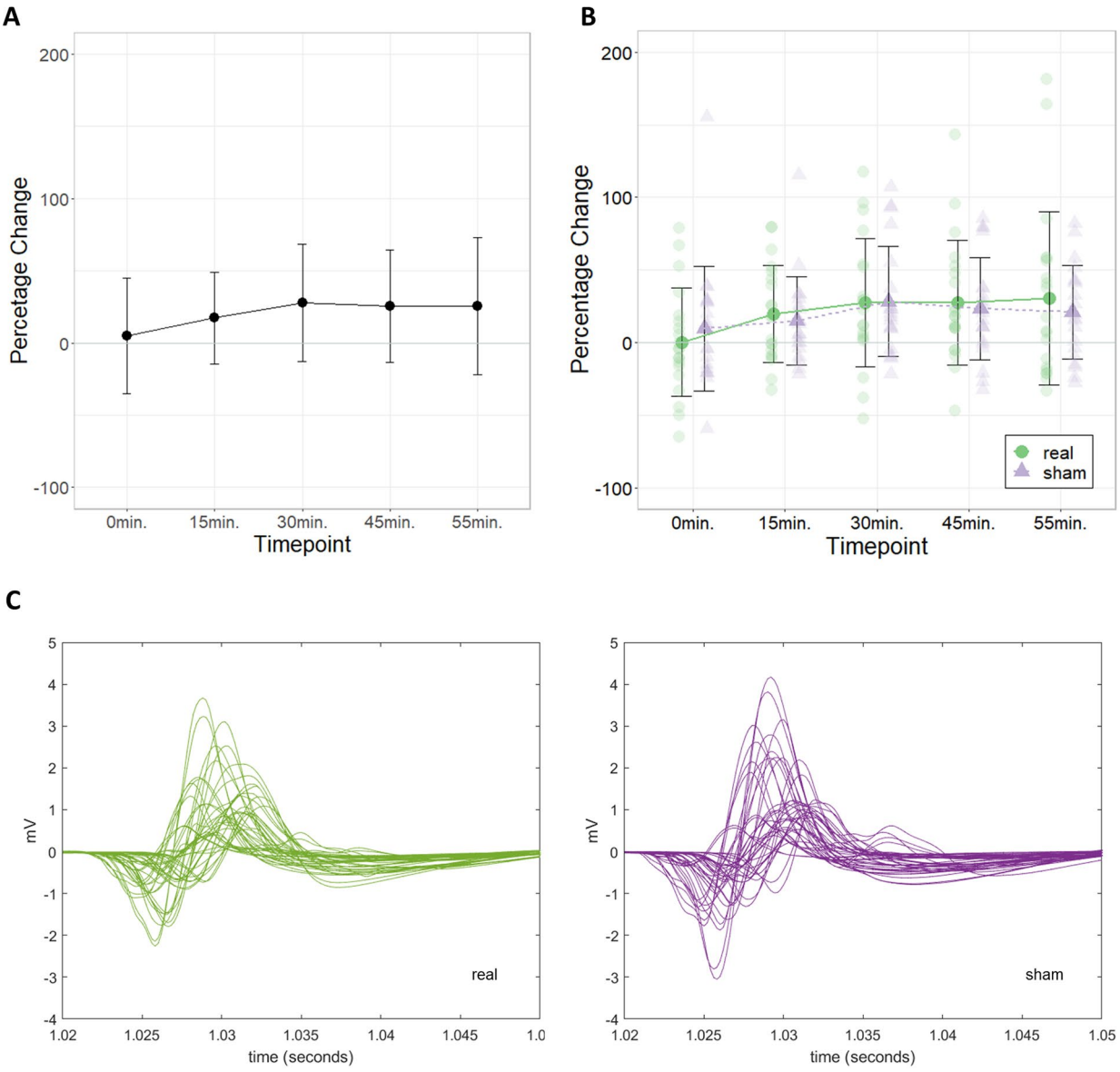

Fig. 2. Group average [+/-SD] percentage change to baseline per timepoint **A)** across both conditions **B**) for each real cTBS and sham cTBS condition **C**) raw MEPs at baseline of real cTBS (left) and sham cTBS (right) condition. *cTBS = continuous theta burst stimulation.

Half of our sample was defined as paradox-responders indicating facilitation post cTBS whereas the other half was defined as responders with expected suppression on MEP amplitudes post cTBS, compared to sham (Fig. 3B). Responder classification rules differ across studies depending on experimental design and parameters estimating cut-off values. Conclusions from responder vs. paradox-responder groups should therefore be interpreted carefully. Previous literature showing peaks of standard cTBS suppression between 10- and 20-minutes post cTBS, declining in magnitude up until 50 min[6] partially supports the visual trend of suppressive effects in the responder group in the current study (Fig. 3A). Based on our regression analysis, neither sex, age, rMT, time of day, nor baseline-latency significantly predicted cTBS responsiveness. Previous research found a robust correlation between late I-wave recruitment and cTBS responsiveness, which showed that participants prone to recruit late I-waves were more likely to show the expected inhibitory effect than participants recruiting early I-waves[13]. Yet, recent work identified a relationship between age, sex and late I-wave recruitment, in which the previously found association of responsiveness and late I-waves was only replicated in adolescent females[42]. We aimed to find a relationship between cTBS response and MEP baseline latencies, which could have been affected by stimulus intensity and D-wave or I-wave recruitment. We did not find a significant predictive effect of baseline-latencies on cTBS responsiveness, but further research might be needed to explore the most adequate classification of responders as well as the relationship of MEP latencies, stimulus intensity and TBS outcomes.

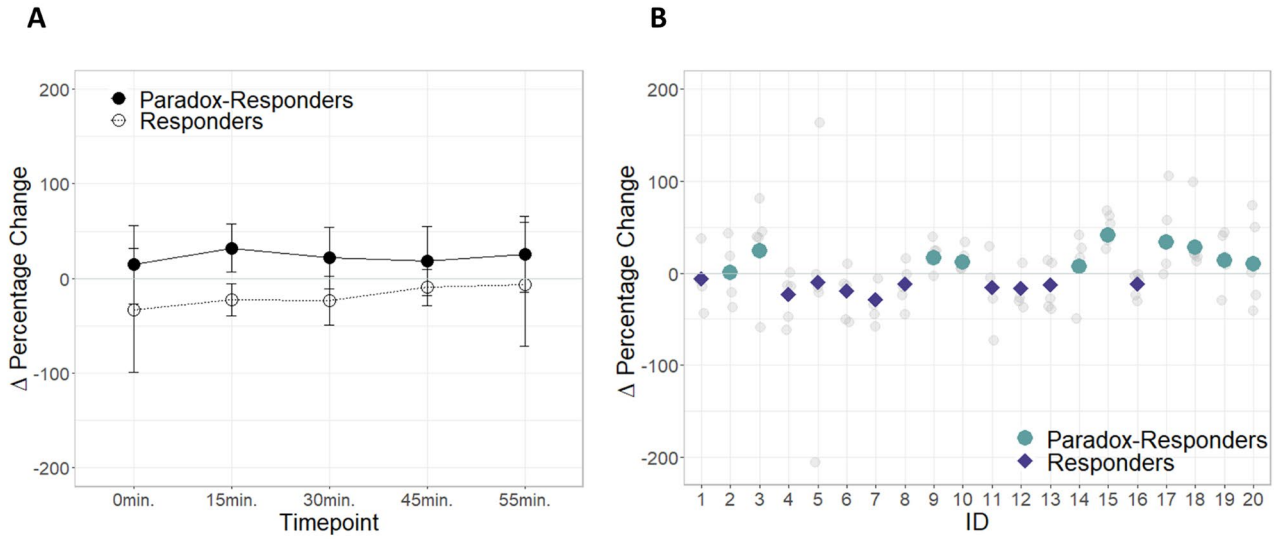

Fig. 3. Subgroup's average difference [+/-SD] in percentage change to baseline values **A**) per timepoint following cTBS stimulation (0, 15, 30, 45 and 55 min) **B**) grand mean across all timepoints per participant dividing the group into Paradox-Responders (positive ΔPC) and Responders (negative ΔPC) accompanied by means per timepoint for each participant. *ΔPC = difference in percentage change; cTBS = continuous theta burst stimulation.

Predicting the responsiveness of TBS outcomes might be dependent on a complex interplay of personal and experimental factors, with latencies providing additional insight into cTBS outcome measures.

The absence of an effect of age on cTBS responsiveness may be due to the current sample consisting of young healthy adults between 19 and 28 years old. The difference in responsiveness within this small age range might not be sufficient to detect any effects of age. Since personal factors like BDNF-genotypes and I-wave recruitment were not in the scope of the current study, we do not know if those could have driven the responsiveness into a certain direction. In future studies, it would therefore be of high importance to consider the interplay of those factors with sex and age in relation to neuromodulatory effects of cTBS.

A limitation of the current study might be the relatively small sample size ($n = 20$). Nevertheless, previous TBS experiments using lower numbers of participants ($n \sim 10$) have found significant suppressive cTBS effects[5,20,43]. These studies might suffer from false positive results (Type-I error) due to a lack of statistical power. Interestingly, our results are in line with the study by Hamada et al., 2012, which revealed no significant effects of TBS in a larger sample size ($n = 50$) and showed opposing patterns of TBS after-effects when dividing the sample into groups of responders and non-responders[13]. However, adequately powered cTBS studies are further needed to draw definite conclusions from TBS effects on cortical excitability. Another limitation of our study is that only participants were blinded to which cTBS condition they were subjected in each session. Due to the 3-D printed spacer attached to the sham coil, and the difference in weight of the coils, it was not possible to blind the experimenter while applying the stimulation. Nevertheless, at the end of the sessions, only one out of 20 participants indicated that they felt a noticeable difference between real and sham cTBS. The level of fatigue was aimed to be kept constant by showing participants nature pictures in the form of a slideshow on the screen in front of them. Still, it is not clear how effective these pictures were in regulating participants' alertness. Future studies might be able to include more objective measurements to capture the level of tiredness by using EEG or eye-tracking techniques to monitor drowsiness and pupil dilation. In addition, considering I-wave recruitment and BDNF genotypes as well as carefully reporting inclusion/exclusion criteria which could interact with additional factors influencing responsiveness will further be necessary to understand how TBS responsiveness is driven not only by external but also internal factors and how responders vs. non-responders can be identified.

Conclusion

In conclusion, the current study showed no suppression of cortical excitability with a 30 Hz cTBS protocol over a 60-minute time window at group level. However, this non-existent effect may be driven by high inter-subject variability marked by differences in responsiveness to cTBS. These outcomes underscore the complexity of TBS effects on cortical excitability and highlight the need for more sham-controlled research to unravel the impact of personal- and experimental factors on neurophysiological mechanisms governing TBS responsiveness. Furthermore, they emphasize the necessity for rigorous validation of tailored cTBS protocols to optimize therapeutic efficacy.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Received: 3 October 2024; Accepted: 26 November 2024
Published online: 05 December 2024

References

1. Klomjai, W., Katz, R. & Lackmy-Vallée, A. Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Annals Phys. Rehabilitation Med.* **58**, 208–213. https://doi.org/10.1016/j.rehab.2015.05.005 (2015).
2. Chung, S. W., Hill, A. T., Rogasch, N. C., Hoy, K. E. & Fitzgerald, P. B. Use of theta-burst stimulation in changing excitability of motor cortex: a systematic review and meta-analysis. *Neurosci. Biobehavioral Reviews*. **63**, 43–64 (2016).
3. Chu, H. T. et al. Efficacy and tolerability of theta-burst stimulation for major depression: a systematic review and meta-analysis. *Prog. Neuropsychopharmacol. Biol. Psychiatry*. **106**, 110168. https://doi.org/10.1016/j.pnpbp.2020.110168 (2021).
4. Li, C. T. et al. Critical role of glutamatergic and GABAergic neurotransmission in the central mechanisms of theta-burst stimulation. *Hum. Brain. Mapp.* **40**, 2001–2009 (2019).
5. Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P. & Rothwell, J. C. Theta burst stimulation of the human motor cortex. *Neuron* **45**, 201–206 (2005).
6. Wischnewski, M. & Schutter, D. J. Efficacy and time course of theta burst stimulation in healthy humans. *Brain Stimul.* **8**, 685–692 (2015).
7. Corp, D. T. et al. Large-scale analysis of interindividual variability in theta-burst stimulation data: results from the 'Big TMS data collaboration'. *Brain Stimul.* **13**, 1476–1488 (2020).
8. Magnuson, J. et al. Neuromodulatory effects and reproducibility of the most widely used repetitive transcranial magnetic stimulation protocols. *PLOS ONE*. **18**, e0286465. https://doi.org/10.1371/journal.pone.0286465 (2023).
9. Ziemann, U. & Siebner, H. R. Inter-subject and inter-session variability of plasticity induction by non-invasive brain stimulation: boon or bane? *Brain Stimulation: Basic. Translational Clin. Res. Neuromodulation*. **8**, 662–663 (2015).
10. Vernet, M. et al. Reproducibility of the effects of theta burst stimulation on motor cortical plasticity in healthy participants. *Clin. Neurophysiol.* **125**, 320–326 (2014).
11. Jannati, A. et al. Test–retest reliability of the effects of continuous theta-burst stimulation. *Front. NeuroSci.* **13**, 447 (2019).
12. Harvey, D. Y. et al. Variability in cTBS aftereffects attributed to the Interaction of stimulus intensity with BDNF Val66Met polymorphism. *Front. Hum. Neurosci.* **15**, 585533. https://doi.org/10.3389/fnhum.2021.585533 (2021).
13. Hamada, M., Murase, N., Hasan, A., Balaratnam, M. & Rothwell, J. C. The role of Interneuron Networks in Driving Human Motor cortical plasticity. *Cereb. Cortex*. **23**, 1593–1605. https://doi.org/10.1093/cercor/bhs147 (2012).
14. Guerra, A., López-Alonso, V., Cheeran, B. & Suppa, A. Variability in non-invasive brain stimulation studies: reasons and results. *Neurosci. Lett.* **719**, 133330 (2020).
15. Guerra, A., López-Alonso, V., Cheeran, B. & Suppa, A. Solutions for managing variability in non-invasive brain stimulation studies. *Neurosci. Lett.* **719**, 133332 (2020).
16. McCalley, D. M. et al. Determining the optimal pulse number for theta burst induced change in cortical excitability. *Sci. Rep.* **11**, 1–9 (2021).
17. Sasaki, T. et al. The intensity of continuous theta burst stimulation, but not the waveform used to elicit motor evoked potentials, influences its outcome in the human motor cortex. *Brain Stimul.* **11**, 400–410. https://doi.org/10.1016/j.brs.2017.12.003 (2018).
18. Vallence, A. M. et al. Inter-and intra-subject variability of motor cortex plasticity following continuous theta-burst stimulation. *Neuroscience* **304**, 266–278 (2015).
19. Di Lazzaro, V. et al. The physiological basis of transcranial motor cortex stimulation in conscious humans. *Clin. Neurophysiol.* **115**, 255–266. https://doi.org/10.1016/j.clinph.2003.10.009 (2004).
20. Goldsworthy, M. R., Pitcher, J. B. & Ridding, M. C. A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. *Clin. Neurophysiol.* **123**, 2256–2263 (2012).
21. Wu, S. W., Shahana, N., Huddleston, D. A. & Gilbert, D. L. Effects of 30 hz theta burst transcranial magnetic stimulation on the primary motor cortex. *J. Neurosci. Methods*. **208**, 161–164 (2012).
22. Ozdemir, Z., Acar, E. & Soysal, A. The effects of 30 hz, 50 hz AND 100 hz continuous theta burst stimulation via transcranial magnetic stimulation on the electrophysiological parameters in healthy individuals. *Ideggyogy Sz.* **74**, 41–49 (2021).
23. Oldfield, R. C. The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia* **9**, 97–113 (1971).
24. Hehl, M., Swinnen, S. P. & Cuypers, K. Alterations of hand sensorimotor function and cortical motor representations over the adult lifespan. *Aging (Albany NY)*. **12**, 4617–4640. https://doi.org/10.18632/aging.102925 (2020).
25. Giustiniani, A. et al. A questionnaire to collect unintended effects of transcranial magnetic stimulation: a consensus based approach. *Clin. Neurophysiol.* **141**, 101–108. https://doi.org/10.1016/j.clinph.2022.06.008 (2022).
26. Opitz, A. et al. Is sham cTBS real cTBS? The effect on EEG dynamics. *Front. Hum. Neurosci.* **8**, 1043 (2015).
27. Boucher, P. et al. Sham-derived effects and the minimal reliability of theta burst stimulation. *Sci. Rep.* **11**, 1–12 (2021).
28. Lüdecke, D. & Lüdecke, M. D. Package 'sjstats'. *Stat. Funct. Regres. Models Version*. **0.17**, 3 (2019).
29. Lüdecke, D., Ben-Shachar, M. S., Patil, I., Waggoner, P. & Makowski, D. Performance: an R package for assessment, comparison and testing of statistical models. *J. Open. Source Softw.* **6** (2021).
30. Fox, J. & Weisberg, S. *An R Companion to Applied Regression* (Sage, 2018).
31. Bates, D., Mächler, M., Bolker, B. & Walker, S. in *Presentation at Potsdam GLMM workshop.*
32. Duque, J. et al. Intermanual differences in movement-related interhemispheric inhibition. *J. Cogn. Neurosci.* **19**, 204–213. https://doi.org/10.1162/jocn.2007.19.2.204 (2007).
33. Duque, J. et al. Kinematically specific interhemispheric inhibition operating in the process of generation of a Voluntary Movement. *Cereb. Cortex*. **15**, 588–593. https://doi.org/10.1093/cercor/bhh160 (2004).
34. Pellicciari, M. C., Miniussi, C., Ferrari, C., Koch, G. & Bortoletto, M. Ongoing cumulative effects of single TMS pulses on corticospinal excitability: an intra- and inter-block investigation. *Clin. Neurophysiol.* **127**, 621–628. https://doi.org/10.1016/j.clinph.2015.03.002 (2016).
35. Hassanzahraee, M., Zoghi, M. & Jaberzadeh, S. Longer Transcranial magnetic stimulation intertrial interval increases size, reduces variability, and improves the reliability of Motor Evoked potentials. *Brain Connect.* **9**, 770–776. https://doi.org/10.1089/brain.2019.0714 (2019).
36. Ridding, M. C. & Ziemann, U. Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *J. Physiol.* **588**, 2291–2304. https://doi.org/10.1113/jphysiol.2010.190314 (2010).
37. Ly, J. Q. M. et al. Circadian regulation of human cortical excitability. *Nat. Commun.* **7**, 11828. https://doi.org/10.1038/ncomms11828 (2016).
38. Turco, C. V., Arsalan, S. O. & Nelson, A. J. The influence of recreational substance use in TMS Research. *Brain Sci.* **10** https://doi.org/10.3390/brainsci10100751 (2020).
39. Noreika, V. et al. Alertness fluctuations when performing a task modulate cortical evoked responses to transcranial magnetic stimulation. *NeuroImage* **223**, 117305. https://doi.org/10.1016/j.neuroimage.2020.117305 (2020).
40. Di Lazzaro, V. et al. I-wave origin and modulation. *Brain Stimul.* **5**, 512–525. https://doi.org/10.1016/j.brs.2011.07.008 (2012).
41. Di Lazzaro, V. et al. Theta-burst repetitive transcranial magnetic stimulation suppresses specific excitatory circuits in the human motor cortex. *J. Physiol.* **565**, 945–950. https://doi.org/10.1113/jphysiol.2005.087288 (2005).

42. Van Dam, J. M., Graetz, L., Pitcher, J. B. & Goldsworthy, M. R. The effects of age and biological sex on the association between I-wave recruitment and the response to cTBS: an exploratory study. *Brain Res.* **1810**, 148359. https://doi.org/10.1016/j.brainres.2023.148359 (2023).
43. Huang, Y. Z., Chen, R. S., Rothwell, J. C. & Wen, H. Y. The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin. Neurophysiol.* **118**, 1028–1032. https://doi.org/10.1016/j.clinph.2007.01.021 (2007).

Acknowledgements
This study was supported by the Research Foundation Flanders grant (G039821N, G1129923N).

Author contributions
J.F. - conceptualization, methodology, formal analysis, investigation, original draft, final manuscript review & editing, visualization, data curation; S.V.H. - software, formal analysis, methodology, final manuscript review & editing, validation; M.N. - methodology, final manuscript review & editing, validation; S.V. - conceptualization, methodology, final manuscript review & editing, formal analysis, supervision; S.P.S. - final manuscript review & editing, supervision, funding acquisition, resources; R.L.J.M. - methodology, final manuscript review & editing, supervision, funding acquisition, resources.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to J.F.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.