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The effect of single and repeated prefrontal intermittent theta burst stimulation on cortical reactivity and working memory

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ABSTRACT

Background: With an increasing interest in the use of theta burst stimulation (TBS) as a cognitive enhancer and a potential therapeutic tool for psychiatric disorders, there is a need to identify optimal parameters of TBS in the prefrontal cortex.
Objective/Hypothesis: This study examined the effect of two blocks of prefrontal intermittent TBS (iTBS) on cortical reactivity and working memory performance, compared to one block of iTBS and sham stimulation. We hypothesized that greater cortical effects would be obtained with two blocks of iTBS.
Methods: Eighteen healthy participants attended three experimental sessions and received either sham, one block or two blocks of iTBS with a 15-min interval. Concurrent transcranial magnetic stimulation with electroencephalography (TMS-EEG) was used to assess the change in cortical reactivity via TMS-evoked potentials. Working memory performance was assessed using the N-back task. Cluster-based permutation statistics and two-way ANOVAs were used for neurophysiological and behavioural data, respectively.
Results: Both single and two blocks of iTBS resulted in a significant increase in the amplitude of TMS-evoked N100 and P200. No significant differences were observed between active conditions in either neurophysiological changes or working memory performance, and both failed to improve working memory performance relative to sham.
Conclusions: Two blocks of iTBS did not result in stronger measured effects as compared to one block of iTBS. Future studies are needed to identify the optimal stimulation pattern in order to achieve a desired effect. It is also important to establish the best approach in quantifying neuromodulatory effects targeting the prefrontal cortex.

Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive technique used to study the physiology of the human brain. Theta-burst stimulation (TBS) is one TMS paradigm, which has a major advantage over conventional repetitive TMS due to its short stimulation duration (20–192s vs > 20min). An intermittent pattern of TBS (iTBS; 2s on, 8s off, 600 pulses) increases the amplitude of motor-evoked potentials (MEPs), while a continuous pattern of TBS (cTBS, 600 pulses) results in the opposite outcome [1]. Efforts have been made to understand the mechanisms involved in the neuroplastic responses to TBS and to enhance the efficacy of TBS in the motor cortex by varying the parameters of stimulation such as intensity [2], frequency [3,4] and number of pulses [5–7]. Studies have found additive after-effects following repeated applications of cTBS [6] and iTBS [7]. However, these dose-dependent findings are not consistent, and reduced [8] or even the opposite effects [5] have been reported depending on the duration of the interval between each block. These findings suggest the

Abbreviations: Ag/AgCl, silver-silver chloride; EEG, electroencephalography; ERP, event-related potential; FDI, first dorsal interosseous; ICA, independent component analysis; LICI, long-interval intracortical inhibition; MEP, motor evoked potential; MNE, minimum norm estimates; rMT, resting motor threshold; SH, sham; SNR, signal-to-noise ratio; (c/i) TBS, (continuous/intermittent) theta burst stimulation; TEP, TMS-evoked potential; TMS, transcranial magnetic stimulation.

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after-effects of TBS may not simply be accumulative. Beyond the motor cortex, there is a paucity of information on the neurophysiological basis of the effects of TBS and the impact of different stimulation parameters on the after-effect. Studies have reported that TBS to the prefrontal cortex can affect cognitive function. For example, prefrontal iTBS has resulted in enhanced working memory performance [9], whereas cTBS has resulted in the opposite outcome [10]. However, such findings are also inconsistent with limited behavioural changes [11,12]. It remains to be determined if repeated application of TBS would promote physiological changes in a dose-dependent manner in the prefrontal cortex, and whether such changes would also result in concurrent behavioural outcomes.

TBS-induced changes in the prefrontal cortex can be probed using concurrent TMS and electroencephalography (TMS-EEG) by examining the changes in TMS-evoked potentials (TEPs) [13]. For instance, iTBS to the prefrontal cortex increases the amplitude of the TMS-evoked N100 [13].

In the present study, we examined whether there were differences in the effects of repeated iTBS stimulation blocks applied to the left prefrontal cortex on cortical reactivity and working memory performance. The experimental procedure involved comparing the effect of two blocks (600 pulses x 2, 15-min interval) of prefrontal iTBS to one block (600 pulses) and sham stimulation on TEPs. The impact of iTBS on working memory performance and task-related electrophysiology (event-related potentials (ERPs)) were also examined. We hypothesized that greater changes in these measures would be obtained with the repeated stimulation blocks/increased number of pulses, and lead to improved working memory performance compared to the application of a single iTBS block or sham stimulation.

Material and methods

Participants

Eighteen healthy subjects volunteered (25.6 ± 7.0 years, 10 female) in the study. All subjects were right-handed according to the Edinburgh Handedness Inventory [14], and average education duration was 16.5 ± 2.3 years. Prior to the experiment, volunteers provided informed consent and were screened with the mini international neuropsychiatric interview (MINI) to confirm no history of mental illness [15]. The experimental procedures were approved by the Alfred Hospital and Monash University Human Research Ethics Committees.

Procedure

Each participant attended 3 sessions with each session at least 1 week apart to avoid carry-over effect. Stimulation conditions were pseudorandomized. The experimental procedures consisted of concurrent recording of EEG during 50 single TMS pulses before (baseline; BL), 5-min post (T5) and 30-min post (T30) iTBS in the prefrontal cortex. Subjects received either sham stimulation (sham iTBS + sham iTBS; SH + SH), a single block of iTBS (sham iTBS + iTBS 600; SH + iTBS) or two blocks of iTBS (iTBS 600 + iTBS 600; iTBS + iTBS) with 15-min interval between each block of iTBS (Fig. 1A). This interval was chosen based on studies that demonstrated the additive effects of TBS when reapplied after 15 min in animals [16] and humans [7,17]. Discomfort level was assessed using 10 cm length numerical rating scale (0: No pain–10: Worst pain) before the first block of iTBS (at BL) and immediately after the second block. The N-back working memory task (2-back and 3-back) was performed before (BL), 15-min post (T15) and 40-min post (T40) iTBS while EEG was recording. Alertness was also measured using 10 cm numerical rating scale (0: Alert–10: Vague) at BL and T40 following working memory tasks to assess attention level.

Transcranial magnetic stimulation

Biphasic TMS pulses (AP-PA current direction in the underlying cortex) were delivered using a figure-of-eight MagVenture B-65 fluid-cooled coil (MagVenture A/S, Denmark) for both single-pulse TMS and iTBS. Resting motor threshold (rMT) was obtained from left motor cortex and identified as the minimum intensity needed to evoke at least 3 out of 6 motor evoked potentials (MEPs) > 0.05 mV in amplitude [18] recorded from the first dorsal interosseous muscles using Ag/AgCl electromyography electrodes. TMS was administered to the left prefrontal cortex at the F1 electrode with the coil positioned at 45° angle relative to midline. This electrode was chosen to minimize the activation of scalp muscles [19], and hence reduce the need for the amount of correction in post-processing of the TMS-EEG data. The edge of the coil was marked on the cap for reliable repositioning of the coil (~5 mm) as has been described as a suitable method when neuronavigation is unavailable [19].

50 single pulses with a 5s interval (10% jitter) were applied to the same left prefrontal region at 120% rMT before and after spaced iTBS. Studies have shown reliable TMS-evoked responses with 50 TMS pulses at supra-threshold intensities [13,20] and a high signal-to-noise ratio (SNR) was obtained particularly for latter peaks (N100 and P200) [13]. Each iTBS block consisted of a burst of 3 pulses (20 ms interval) repeated every 200 ms for 2s with an 8s break for a total of 600 pulses, given at an intensity of 75% rMT. The rMT was measured on each session, and the average intensity for each condition was as follows: SH + SH: 54.28 ± 6.5%; SH + iTBS: 54.39 ± 6.6%; and iTBS + iTBS: 54.33 ± 6.9%. The rMT for each individual at each session can be found in Supplementary Material, Table S1. The average of coefficient of variation for rMT between session was 1.14% (range: 0–3.03%). For sham iTBS, the coil was rotated 90° around the handle so that the right wing was touching the F1 electrode. In this position, the magnetic field is tangential to the scalp and does not result in cortical stimulation.

Working memory performance (N-back tasks)

Participants performed 5min of both the 2-back and 3-back task in a pseudorandomised (e.g. either 2-back followed by 3-back or vice versa) at three time points during each experimental session. A random series of white letters from A to J were presented for 500 ms every 2000 ms on a black screen in a consecutive manner. Participants were required to respond with a button press when the presented letter corresponded to the letter that appeared either 2 (Fig. 1B) or 3 trials before (Fig. 1C). Each task contained 130 trials with 25% targets. Working memory performance was assessed using *d* prime (*d'*) and accurate reaction time. *d'* quantifies performance with regards to hits and false alarms ($d' = Z$ (hit rate)$-Z$ (false alarm rate)) [21].

EEG recording and data preprocessing

A detailed procedure for the recording and preprocessing of the EEG data can be found in Supplementary Material, Methods section 1 & 2. Briefly, EEG was recorded using 48 TMS-compatible Ag/AgCl electrodes on a 64-channel EEG cap, referenced to CPz and grounded to FPz. The sampling rate for TMS-EEG and N-back task were 10,000 Hz and 1000 Hz, respectively. Electrode impedance was kept below 5 kΩ and white noise was used to mask TMS click sound.

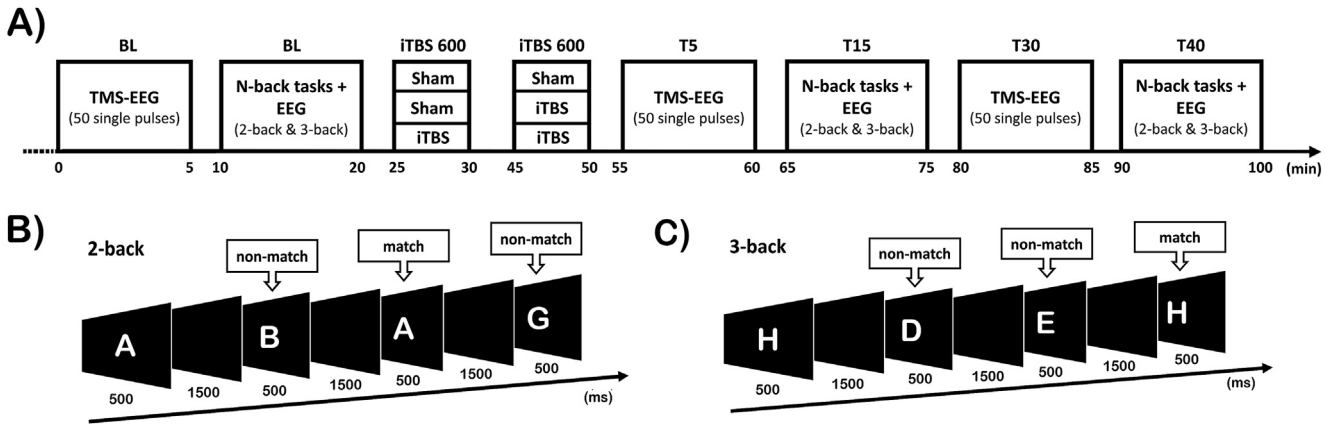

Fig. 1. Experimental design of the study. (A) Combined transcranial magnetic stimulation and electroencephalography (TMS-EEG) and N-back tasks were obtained at baseline (BL) before intermittent theta burst stimulation (iTBS). Two blocks of sham and/or real iTBS was given at 15 min interval, and post-iTBS measures were obtained twice (TMS-EEG – T5 and T30; N-back – T15 and T40). (B–C) Schematic diagram of N-back tasks illustrating match trials for either 2-back or 3-back task. Letters were presented for 500 ms with 1500 ms interval in-between.

TMS-EEG data

Data were epoched around the TMS pulse, baseline corrected, and the TMS pulse artefact was removed and interpolated. Data were down sampled and were visually inspected for excessive noise in the signal. Two rounds of independent component analysis (FastICA) was used to remove non-neural components using TESA toolbox [22]. Filters were applied between the two FastICAs.

N-back EEG data

Continuous data were filtered, epoched around correctly encoded and maintained trials and baseline corrected. Data were concatenated across epochs from three time points (BL, T15, T40) and underwent preprocessing steps with only one round of ICA.

For all EEG data, any removed channels were interpolated and data were re-referenced to common average reference. The averaged evoked potentials were analysed separately for each time point (TMS-EEG: BL, T5, T30; N-back: BL, T15, T40).

TMS-evoked potentials (TEPs) and event related potentials (ERPs) during N-back tasks

Both TEPs and ERPs were analysed using cluster-based permutation statistics at a global scalp level. Pre-determined time window for each peak of interest was used for TEPs [N45 (35–55 ms), P60 (55–80ms), N100 (95–135ms) and P200 (160–240ms)] and ERPs [N100 (75–125ms), P150 (125–175ms), N200 (190–260ms) and P300 (280–380ms)], and data were averaged across time prior to the statistical comparisons across the scalp. These peaks are commonly described in the literature (TEPs [13,20,23,24]; ERPs [25–27]). The waveforms were graphically represented using the average of 3 fronto-central electrodes (FC1, FCz and FC2), as these electrodes were close to the site of stimulation, and electrodes in contact with the MagVenture coil contain unwanted artefacts [19]. Source estimation was computed using Brainstorm software [28] (Supplementary Material, Methods section 3). An SNR analysis was conducted on these electrodes to verify the number of pulses included in the study (~48 pulses) was adequate. The SNR was determined by dividing the amplitude of peaks by the standard deviation (SD) of the signals in the pre-stimulus duration (−500 to −50 ms) [13,29].

Statistics

Statistical analysis was performed in SPSS (Version 22) and Matlab. Non-parametric cluster-based permutation statistics were used for the analysis of all electrophysiological data at a global scalp level, which provides a robust method of controlling for multiple comparisons in space (EEG electrodes) and time [30]. Within-condition comparisons were first assessed for each TBS condition over time (between BL and T5/T30 for TMS-EEG, between BL and T15/T40 for N-back EEG). Between-condition comparisons were then investigated using the change-from-baseline scores following iTBS (post–pre; Δ). Monte Carlo *p*-values were calculated on 2500 randomizations and a value of $p < .05$ was used as the cluster statistical significance for two or more neighbouring electrodes for all analyses, controlling for multiple comparisons across space and time ($p < .025$; two-tailed test).

For behavioural measures, repeated measure 3 (time: BL, T15 and T40) x 3 (stimulation conditions – SH + SH, SH + iTBS and iTBS + iTBS) analysis of variance (ANOVAs) were conducted for working memory performance in *d'* and accurate reaction time, and 2 (time: BL and T40) x 3 (stimulation conditions) ANOVAs for numeric ratings. Pearson's correlations were used to assess the relationship between the changes in TMS-evoked activities, N-back task related electrophysiology and working memory performances.

Results

Tolerability of iTBS

Subjects reported no side-effects, such as headaches or dizziness. The average pain rating (0: No pain–10: Worst pain) in the stimulated area for each condition were as follows (pre & post): SH + SH: 0.14 ± 0.3 & 0.22 ± 0.6; SH + iTBS: 0.38 ± 0.8 & 0.54 ± 1.5; iTBS + iTBS: 0.73 ± 1.3 & 0.58 ± 0.9. Repeated measures ANOVA showed no significant main effect of condition ($F_{2,34} = 1.958$, $p = .157$), time ($F_{1,17} = 0.072$, $p = .791$), nor interaction ($F_{2,34} = 0.370$, $p = .694$). Overall, iTBS was safe with negligible pain rating.

Single-pulse TMS

Fig. 2 illustrates an overview of TEP waveforms following single-pulse TMS over left prefrontal cortex (F1 electrode, marked as 'X' on

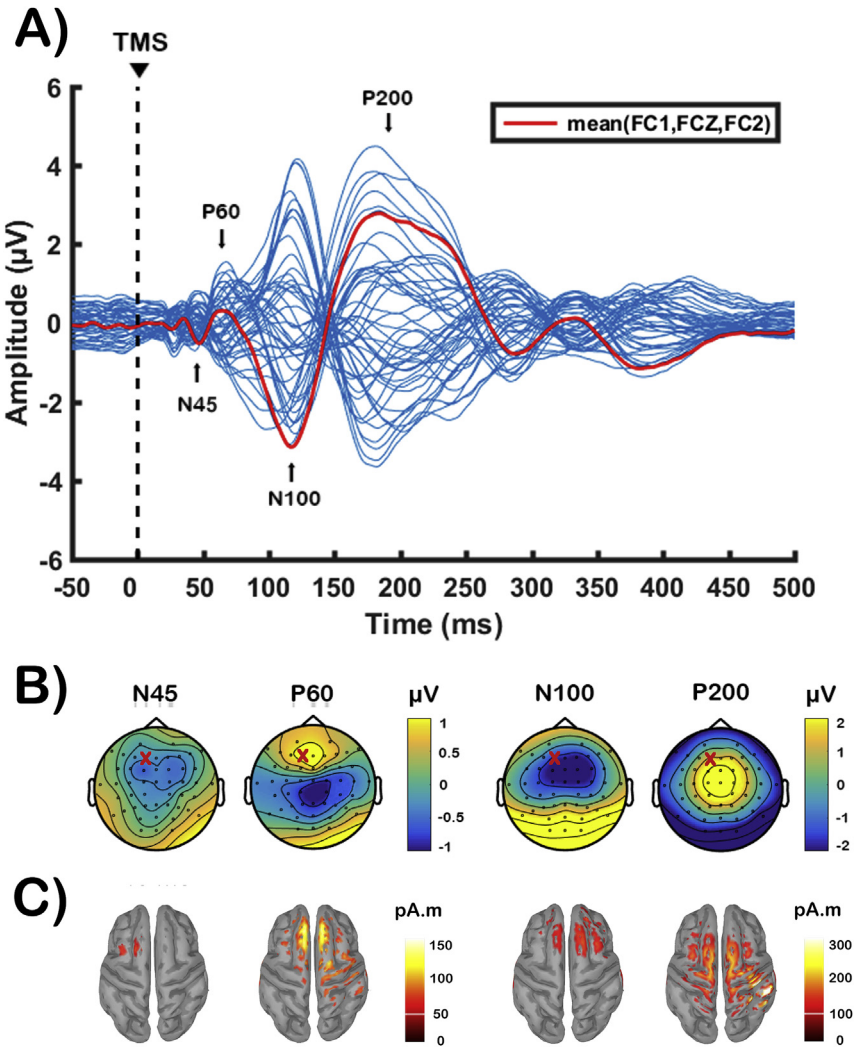

Fig. 2. Transcranial magnetic stimulation (TMS)-evoked potentials following single-pulse stimulation over left prefrontal cortex (F1 electrode) before theta-burst stimulation (data combined for all stimulation conditions at baseline). (A) Butterfly plot of all electrodes with peaks of interest (N45, P60, N100, P200) shown in text. The red line indicates the waveform obtained from the average of three fronto-central electrodes (FC1, FCz, FC2) for graphical representation. (B) Voltage distribution and (C) Minimum Norm Estimates (MNEs) of the source level activity at the cortex for each peak. 'X' on topoplots represent the stimulation site. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

topoplots) and the estimated source of the peaks of interest (N45, P60, N100 and P200). The waveform, scalp topography and source estimation were consistent with other TMS-EEG studies in the prefrontal cortex [13,20,24].

The SNR of single-pulse TMS included in the analyses can be found in the Supplementary Material, Table S2. Qualitatively N45 and P60 peaks displayed a moderate-to-good SNR, but N100 and P200 exhibited excellent SNR.

The effect of spaced iTBS on TMS-evoked activity

The aftereffects of iTBS were first assessed by comparing the amplitude of TEPs over time in each stimulation condition. Testing for an N100 effect in the pre-defined latency range (as stated in section 2.7), the cluster-based permutation tests revealed significant differences between pre-iTBS (BL) and 5-min post iTBS (T5) following SH + iTBS ($p = .003$; Fig. 3A), and between BL and 30-min post iTBS (T30) following iTBS + iTBS ($p = .008$; Fig. 3B), which were observed in fronto-central sensors.

For P200, significant differences were found between BL and T5 ($p = .007$), and between BL and T30 for SH + iTBS ($p = .023$), whereas a significant difference was found between BL and T5 ($p = .011$) and a trend between BL and T30 ($p = .032$) for iTBS + iTBS. These differences were most pronounced over fronto-central sensors. No significant changes were observed in SH + SH condition for any peaks ($p > .025$; Fig. 3C), and no other peaks showed any significant changes in any of the conditions (all $p > .025$).

For comparison across conditions, we calculated the iTBS-induced changes in TEP amplitude by subtracting pre-signals (BL) from post-signals (T5 and T30) (change-from-baseline scores; Δ) and compared Δ between each stimulation condition. We found that ΔN100 and ΔP200 were larger following SH + iTBS than SH + SH (N100: SH + iTBS > SH + SH − T5, $p = .006$; T30, $p = .012$; P200: SH + iTBS > SH + SH − T30, $p = .012$), which was observed in fronto-central sensors (Fig. 3D). The comparisons between iTBS + iTBS and SH + SH in ΔN100 and ΔP200 were trending towards significance (N100: iTBS + iTBS > SH + SH − T5, $p = .048$; T30, $p = .053$; P200: SH + iTBS > SH + SH − T5, $p = .052$). No significant differences in iTBS-induced change were observed between SH + iTBS and iTBS + iTBS conditions (all $p > .025$).

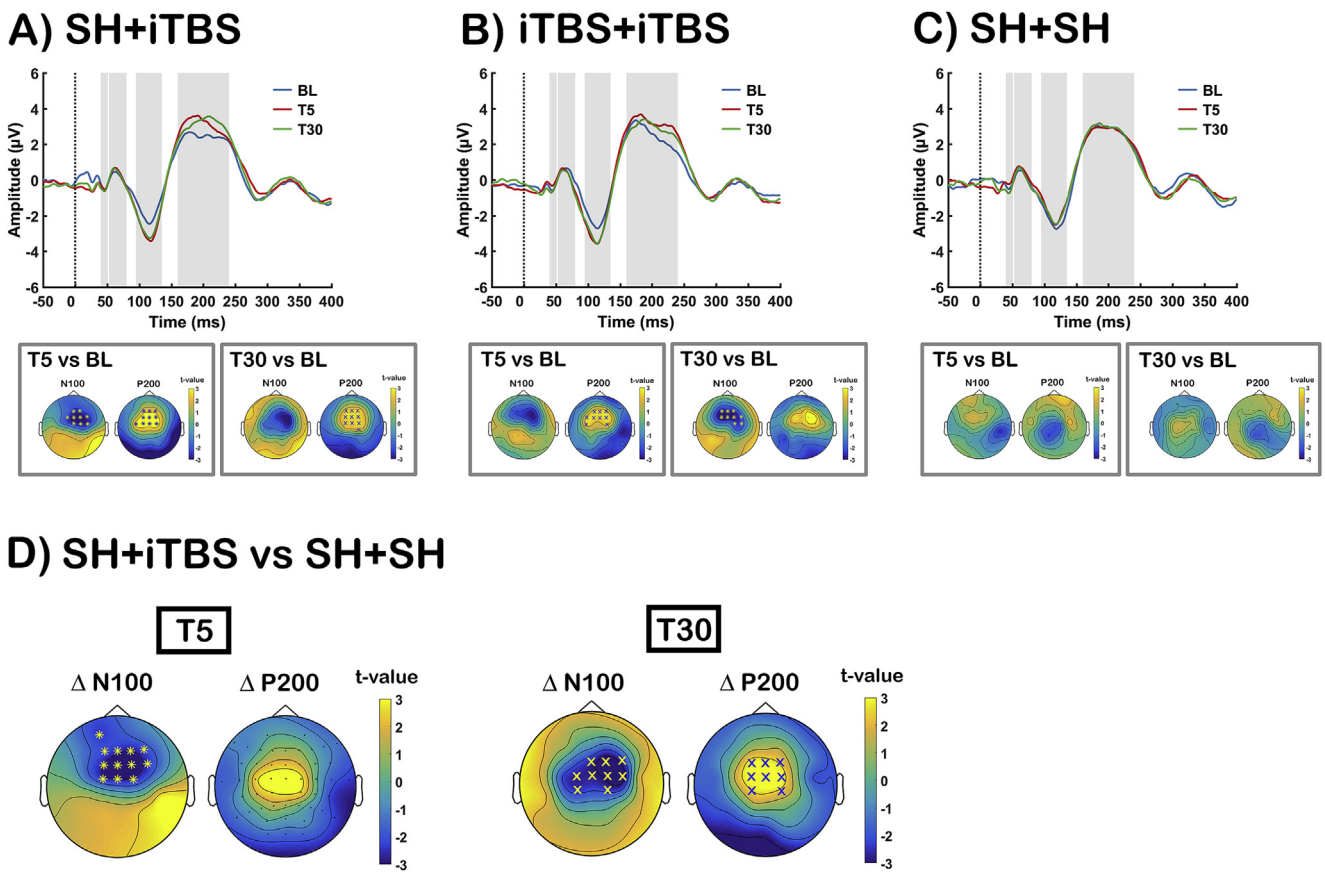

Fig. 3. Modulation of cortical activity assessed via transcranial magnetic stimulation (TMS)-evoked potentials (TEPs) following different intermittent theta burst stimulation (iTBS) conditions (A: SH + iTBS; B: iTBS + iTBS; C: SH + SH). Grand average TEP waveforms at BL (blue), T5 (red) and T30 (green) using the average 3 fronto-central electrodes (FC1, FCz and FC2). Scalp maps represent t-values for comparison between time points. (D) Comparison between iTBS-induced Δ N100 and Δ P200 between SH + iTBS and SH + SH conditions at T5 and T30. Asterisks and 'X's on topoplots indicate significant sensors between comparisons (cluster-based statistics, $^{*}p < .01$, $^{X}p < .025$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

A power analysis was performed on ΔN100 and ΔP200 to determine if the current study was powered to detect subtle differences between two active conditions using G*Power software [7]. A large effect size was required for the detection of differences, which was not present in the comparison between active conditions (Supplementary Material, Fig S1). In order to obtain power of 0.80 with an alpha of 0.025 for the active condition comparisons, 865 and 495 subjects would be required for ΔN100 at T5 and T30, respectively. For ΔP200, 557 subjects would be needed for T5 and 76 for T30.

The change in N100 and P200 were examined at a single-subject level to determine whether variability in response to stimulation had an impact on the TMS-EEG results (Supplementary Material, Fig S2). The majority of the subjects responded in one direction (i.e. increased amplitude) following active iTBS conditions whereas such specific directional change was not present following the sham condition (Supplementary Material, Fig S3 & Table S3).

The effect of spaced iTBS on neurophysiology during N-back tasks

During the 2-back task, we were unable to detect any significant changes in event-related potentials (ERPs) following any iTBS condition (all $p > .025$) (Supplementary Material, Fig S4). However, during the 3-back task, the amplitude of N200 increased following both SH + iTBS ($p = .009$, frontal; $p = .022$, posterior; Fig. 4A) and iTBS + iTBS ($p = .008$, frontal; Fig. 4B) at T15. No significant differences were seen in any other peaks, and SH + SH condition did not alter the amplitude of any peak (Fig. 4C) (all $p > .025$).

For the comparison across conditions, ΔN200 was larger following both SH + iTBS ($p = .002$) and iTBS + iTBS ($p = .016$) compared to SH + SH at T15, and the differences were most pronounced over fronto-central sensors (Fig. 4A and B). No significant differences were observed between SH + iTBS and iTBS + iTBS in any peak (all $p > .025$).

The link between TEP N100 and ERP N200

TMS-evoked N100 [13,23,31] and task-related N200 [32,33] have been linked to inhibitory processing. These peaks were strongly modulated by active iTBS conditions, and therefore, we conducted exploratory correlation analyses between TMS-evoked ΔN100 (T5 & T30) and ERP ΔN200 during 3-back task (T15 & T40). Significant correlations were observed between the two different measures in SH + iTBS condition (T5/T15: $r = 0.663$, $p = .003$; T30/T40: $r = 0.607$, $p = .008$), a trend toward significance in iTBS + iTBS (T5/T15: $r = 0.430$, $p = .075$) and no significance in sham condition ($p > .05$) (Supplementary Material, Fig S5).

The effect of spaced iTBS on working memory performance

We first conducted order effect analysis to confirm the effectiveness of the counter-balancing of stimulation conditions. One-way repeated measures ANOVA showed no significant session

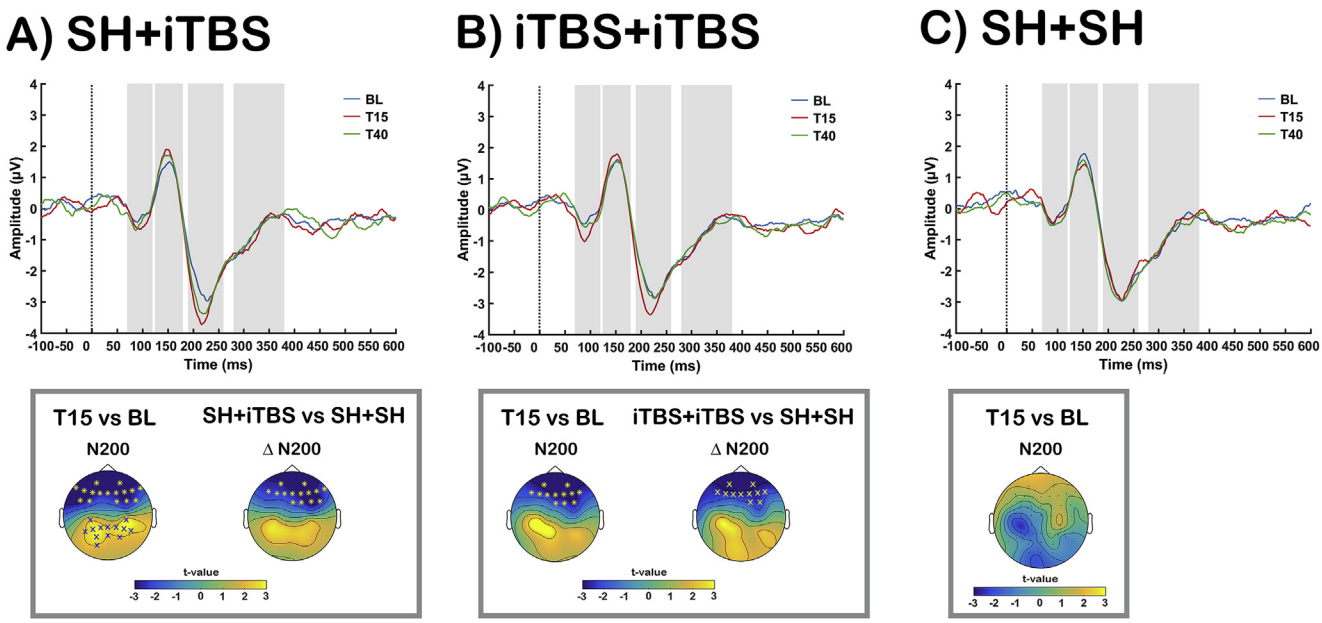

Fig. 4. Effects of different intermittent theta-burst stimulation (iTBS) conditions (A: SH + iTBS; B: iTBS + iTBS; C: SH + SH) on the event-related potentials during 3-back task. Grand average waveform of event-related potentials (ERPs) at BL (blue), T15 (red) and T40 (green) using the average 3 fronto-central electrodes (FC1, FCz and FC2), with scalp maps representing t-values for comparison between time points and/or conditions. Asterisks and 'X's on topoplots indicate significant sensors between comparisons (cluster-based statistics, $^{*}p < .01$, $^{X}p < .025$). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

effects in either accuracy (d'–2-back: $F_{2,34} = 0.477$, $p = .625$; 3-back: $F_{2,34} = 0.180$, $p = .836$) or accurate reaction time (2-back: $F_{2,34} = 0.758$, $p = .476$; 3-back: $F_{2,34} = 0.259$, $p = .774$) for both working memory tasks at baseline measure.

Mean d', accurate reaction time and the effect sizes for 2-back and 3-back tasks before and after each stimulation condition can be found in Supplementary Material, Table S4 and S5. A significant overall improvement in d' was seen for both the 2-back (Fig. 5A) and 3-back (Fig. 5B) tasks with no differences between the groups (no main effect of condition or interaction). In addition, no significant effect of iTBS on alertness rating was observed (Fig. 5C; Supplementary Material, Table S6).

Discussion

This study investigated electrophysiological and cognitive effects of the repeated application of iTBS over the left prefrontal cortex. The findings demonstrate that two blocks of iTBS with a 15-min interval does not increase iTBS-induced changes in cortical properties measured via evoked potentials and working memory performance compared to a single block of iTBS. We also found that changes in working memory performance following a single and two blocks of iTBS were related to changes in cortical activity, however, the size of these performance changes was subtle. Although our observation suggests that the two active stimulation conditions produced similar effect over the prefrontal cortex in healthy individuals, methodological limitations need to be accounted for when interpreting the results.

Effects of iTBS on TMS-evoked activity

Within-group comparisons revealed both one block (SH + iTBS) and two blocks of iTBS (iTBS + iTBS) over the prefrontal cortex increased N100 TEP amplitude. Increasing the size of the N100 TEP following iTBS is in line with our previous study in the prefrontal cortex [13], and TMS-EEG studies of iTBS in other brain regions [34,35], which may represent increased cortical inhibition [13,36–39]. We also observed increased P200 following both active iTBS conditions, which is consistent with our previous study [13]. The underlying physiology of this component is still unknown and further characterization is required. Examination of ΔN100 and ΔP200 in each individual did not reveal substantial difference in the number of responders (i.e. increased N100 & P200) to iTBS + iTBS compared to SH + iTBS (Supplementary Material, Table S3). It is possible that the comparison between iTBS + iTBS and SH + SH did not reach significance due to small, non-specific directional change

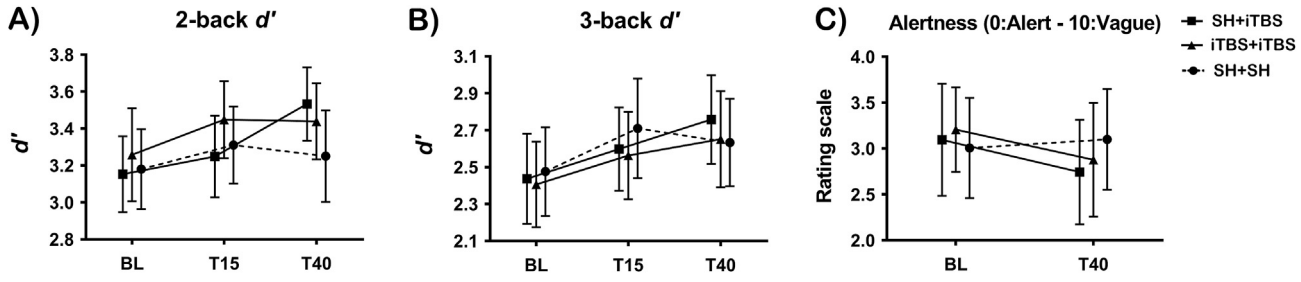

Fig. 5. Working memory performance assessed by d' at BL, T15 and T40 in different intermittent theta-burst stimulation (iTBS) conditions during (A) 2-back and (B) 3-back tasks, and (C) alertness level. Error bars indicate standard error of means (SEM).

in the amplitude of these peaks following sham stimulation, and therefore, larger effects may have been necessary following active stimulation conditions to observe statistical differences.

The lack of any noticeable difference between cortical changes following SH + iTBS and iTBS + iTBS suggests cortical reactivity does not increase with dose.

Effects of iTBS on neurophysiology during N-back tasks

The N200 ERP during working memory has been associated with cognitive control [40], attention [25] and inhibition [33]. We observed a significant increase in N200 amplitude during the 3-back task following both active iTBS conditions. A similar pattern was observed during the 2-back task, but it was not significant. During the 3-back task, within condition comparison showed that N200 amplitude increased following both SH + iTBS and iTBS + iTBS at T15, and both stimulation conditions resulted in an ERP ΔN200 that was larger than sham stimulation. No apparent differences between two active conditions again indicate no dose-dependent effect following two blocks of stimulation. The positive correlations between TEP ΔN100 and ERP ΔN200 suggest the involvement of similar mechanism, possibly cortical inhibition, and N100 in this study is therefore unlikely to be attributed to auditory processing as described in a previous study [41].

Effects of iTBS on working memory performance

Despite no observable change in electrophysiology during 2-back task, we found improvement in working memory performance (*d'*) over time regardless of stimulation condition. This suggests that the enhancement may have been due to practice effects. Previous studies have shown working memory improvements following iTBS over prefrontal cortex [9] or deficits following cTBS [10] in the 2-back condition. In this study, similar yet non-significant behavioural outcomes were seen in the 3-back task, which were accompanied by robust changes in cortical neurophysiology following active stimulation conditions. This is in contrast with a previous study which demonstrated particularly large effect size of approximately $d = 1.5$ [9]. In the current study, the effect sizes for 3-back task following SH + iTBS was $g = 0.43$ (Supplementary Table S5), which is distinctly lower than aforementioned study. However, the effect size in this study is comparable to a recent meta-analysis on working memory performance improvement following non-invasive brain stimulation (confidence interval $g = 0.112$ and 0.395), particularly in healthy individuals [42]. Therefore, more study is needed to verify the effectiveness of iTBS in enhancement of cognitive performance.

Even though changes in working memory performance were not statistically different between active and sham conditions, significant correlations were found between Δ*d'* and both TMS-evoked ΔN100 and 3-back task related ΔN200 (increased amplitude related to improved accuracy) only in the SH + iTBS condition (Supplementary Material, Fig S6 & S7). It is possible that when neurophysiological change is strong enough, changes in behavioural response can be observed. However, the variability of neurobiological response may be washing out what is likely a more subtle behavioural impact of stimulation.

Possible reasons for the negative outcome

Overall, stronger effects were not observed following two blocks of iTBS in the prefrontal cortex compared to one block of iTBS. Homeostatic mechanisms may be a possible explanation for the results as have been reported in motor cortex studies using iTBS at varying intervals [8,43]. The lack of differences between SH + iTBS and iTBS + iTBS could result from the second iTBS block having less novelty when preceded by another block after 15 min, thus leading to habituation of the measured effects. Similar outcomes using two blocks of iTBS have been demonstrated in a rat model [16] and in the human motor cortex [7] where the same duration of stimulation break (15-min) was used. However, markedly increased dose-dependent effects were observed following the third application of iTBS (3 × iTBS, 15-min break) in these studies. Future studies should consider extending the number of iTBS blocks to investigate whether more efficacious after-effects of stimulation can be obtained. Despite different stimulation paradigms, other studies have shown more robust changes following two blocks of cTBS (2 × 600 pulses; 10-min interval) over motor cortex [6] and parietal cortex (15-min interval [17]). Differences in stimulation parameters limit a systematic comparison between studies. It is unclear what impact the interval between the doses has on the after-effects of TBS in the prefrontal cortex, and the possibility that the effects of single block of iTBS are short lasting and therefore may not accumulate with 15-min interval should not be disregarded. On the other hand, enhancement of previously saturated LTP was observed only when TBS was repeated 1 h or longer, but not when shorter intervals were used in rats [44]. It remains to be determined what interval would be optimal and how many blocks of stimulation would be sufficient in order to obtain the most robust outcome. Another possible reason for not observing a dose-dependent effect following iTBS + iTBS is the limited time point of measurement (30 min for TMS-EEG, 40 min for N-back task) which may have been too short to capture the full effect. Monte-Silva and colleagues [45] observed elevated amplitude of MEPs for more than 24 h following two blocks of anodal tDCS (3 or 20 min interval) compared to a single block. The effects within 120 min were similar, if not smaller than a single block of stimulation. Therefore, future studies should investigate the extended effects of repeated application of iTBS in the prefrontal cortex. Finally, it is also possible that the statistical approach adopted in this study may have limited the sensitivity to detect subtle differences between conditions, particularly between two active stimulation conditions. The current method is widely utilised in the electrophysiological studies and has a very high precision, however the sensitivity may be low and requires larger sample sizes as indicated in the power analysis, trials per TEP/ERP or larger effect sizes in order to observe significant differences [46]. Establishing an optimal method of quantifying neuromodulatory changes targeting the prefrontal cortex would benefit future studies.

Limitations

There are several limitations of the study. Even though the experimental protocol was sham-controlled, it was not blinded. It is currently difficult to truly blind both the TMS administrator and the subject. In addition, only one baseline recording was performed using TMS-EEG with limited number of trials which may have affected the ability to detect any difference in the early TEP components due to lower signal-to-noise ratio compared to late TEPs. Results have been consistent with our previous studies, however, increasing the number of TMS-EEG pulses may shed a new light on the after-effects of iTBS. It has been shown that TMS click sound can induce N100-P200 component, even in the presence of noise masking [41]. As we used a repeated measures, possible auditory artefacts would be consistent across time, and any change in TEPs is likely to be attributed to neural activity. The sham condition did not

alter the overall amplitude of this component, which confirms the validity of the results. Increasing the number of EEG channels would also provide better spatial resolution. The use of neuronavigation, which was not feasible in this study, could improve the localisation of stimulation site between sessions. Lastly, despite its common use in EEG studies, the statistical method used in this study may not be sensitive to subtle differences between conditions given small number of participants.

Conclusions

In conclusion, two blocks of iTBS did not yield stronger measured effects as compared to one block of iTBS within 40-min post stimulation. The results, however, should be interpreted with caution due to methodological and analytical limitations.

Disclosures and conflict of interests

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.brs.2018.01.002.

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