Cerebral Cortex, December 2016;26: 4563–4573

doi:10.1093/cercor/bhw193
Advance Access Publication Date: 23 September 2015
Original Article

ORIGINAL ARTICLE

# Enhancement of Working Memory and Task-Related Oscillatory Activity Following Intermittent Theta Burst Stimulation in Healthy Controls

Kate E. Hoy[1], Neil Bailey[1], Marco Michael[1], Bernadette Fitzgibbon[1], Nigel C. Rogasch[2], Takashi Saeki[1] and Paul B. Fitzgerald[1]

[1]Monash Alfred Psychiatry Research Centre, The Alfred and Monash University, Central Clinical School, Melbourne, VIC 3004, Australia and [2]Monash Clinical and Imaging Neuroscience, School of Psychological Science and Monash Biomedical Imaging, Monash University, Melbourne, VIC 3800, Australia

Address correspondence to Dr Kate Hoy, Monash Alfred Psychiatry Research Centre, Level 4, 607 St Kilda Road, Melbourne, VIC 3004, Australia. Email: kate.hoy@monash.edu

## Abstract

Noninvasive brain stimulation is increasingly being investigated for the enhancement of cognition, yet current approaches appear to be limited in their degree and duration of effects. The majority of studies to date have delivered stimulation in “standard” ways (i.e., anodal transcranial direct current stimulation or high-frequency transcranial magnetic stimulation). Specialized forms of stimulation, such as theta burst stimulation (TBS), which more closely mimic the brains natural firing patterns may have greater effects on cognitive performance. We report here the findings from the first-ever investigation into the persistent cognitive and electrophysiological effects of intermittent TBS (iTBS) delivered to the left dorsolateral prefrontal cortex. In 19 healthy controls, active iTBS significantly improved performance on an assessment of working memory when compared with sham stimulation across a period of 40 min post stimulation. The behavioral findings were accompanied by increases in task-related fronto-parietal theta sychronization and parietal gamma band power. These results have implications for the role of more specialized stimulation approaches in neuromodulation.

**Key words:** cortical oscillations, dorsolateral prefrontal cortex, theta burst stimulation, working memory

## Introduction

Over the last 10 years research into the use of noninvasive brain stimulation for the enhancement of cognition has dramatically increased (Brunoni and Vanderhasselt 2014; Coffman et al. 2014). Studies have found improvements in performances on tasks ranging from working memory to mental arithmetic and even complex decision making (Hecht et al. 2010; Hauser et al. 2013; Hoy et al. 2013). The majority of this research has used either anodal transcranial direct current stimulation (a-tDCS) or high-frequency transcranial magnetic stimulation (HF-TMS) (Brunoni and Vanderhasselt 2014; Luber and Lisanby 2014). Despite the widespread interest and excitement in this area, results from recent meta-analyses indicate that these standard approaches may be limited in both their degree and duration of effects on cognition—particularly in healthy controls (Brunoni and Vanderhasselt 2014; Luber and Lisanby 2014). Focus is now turning to more specialized forms of stimulation and while the majority of this focus has been on alternative forms of tDCS, there are also TMS approaches that, in theory, could prove more effective.

TMS produces a magnetic field that passes into the brain and stimulates electrical activity causing brain cells to fire; when given at high-frequency (i.e., above 1 Hz) TMS can increase brain activity (Fitzgerald et al. 2006). This increase in cortical excitability is one possible mechanism by which TMS is able to enhance cognition. (Rossi and Rossini 2004; Brunoni and Vanderhasselt 2014). Alternative approaches to the delivery of TMS, such as theta burst stimulation (TBS) which is thought to more robustly alter cortical excitability by mimicking the brain's natural firing patterns, may have greater potential than the standard approach. TBS involves the provision of TMS pulses in very short bursts at high frequency (usually ~ 50 Hz). These short bursts are repeated at an interval of 200 ms so that the bursts are being applied at a frequency of 5 Hz. Intermittent TBS (iTBS) has been shown to be excitatory and involves applying TBS in 2-s trains every 10 s (2 s of stimulation followed by 8 s of rest) (Huang et al. 2005). In the motor cortex, iTBS has been shown to induce greater and longer lasting effects on cortical excitability than that seen with standard HF-TMS (Huang et al. 2005; Di Lazzaro et al. 2008). Animal research investigating the mechanism of action of iTBS suggests that this lasting enhancement of cortical excitability is achieved primarily by reducing the inhibitory control of pyramidal cells, leading to increased excitatory output and the induction of plastic like changes in the brain (Benali et al. 2011). This is consistent with research in humans showing that the after effects of iTBS in the motor cortex are dependent on the function of NMDA receptors, which are integral to neuroplastic plastic processes such as long-term potentiation (LTP) (Hrabetova et al 2000; Huang et al. 2007). The superior effects of iTBS, when compared with HF-TMS, are thought to be due to the more "naturalistic" pattern of stimulation provided with iTBS which is based on in vivo patterns of pyramidal neuronal firing associated with LTP induction (Oberman et al. 2011). Therefore, iTBS delivered to a more cognitively relevant brain region (i.e., the dorsolateral prefrontal cortex [DLPFC]), may be able to produce more robust cognitive enhancement than that seen with the standard approaches due to its superior ability to enhance cortical excitability. To the best of our knowledge, there has been no research to date examining the persistent cognitive effects of iTBS to the DLPFC in healthy controls.

The current study investigated the effect of iTBS to the left DLPFC, when compared with sham stimulation, on the post-stimulation performance of a working memory task. We hypothesized that iTBS would result in enhanced working memory performance over time compared with sham. We also explored the effect of iTBS on task-related cortical oscillatory activity, specifically theta and gamma activity in the fronto-parietal network as these are strongly implicated in working memory (Baddeley 2003; Lisman 2010).

## Materials and Methods

### Participants

Twenty healthy control participants were recruited into the study. One participant's data were excluded due to equipment malfunction leaving 19 participants with complete datasets (see Table 1 for demographic data). Criteria for exclusion included a history of any psychiatric or neurological illness, seizure, any serious medical conditions, or current pregnancy. Suitability was determined via interview which included administration of a TMS Safety Screen and the Mini International Neuropsychiatric Interview (MINI)

**Table 1** Participant demographics

| | |
|---|---|
| Gender (f/m) | 9/10 |
| Handedness (r/l) | 19/0 |
| Age | 22.16 ± 2.93 |
| Education (undergraduate/postgraduate) | 17/2 |

(Sheehan et al. 1997). Ethical approval was granted by Monash University and the Alfred Hospital ethics committees. Written consent was obtained from all participants prior to the commencement of the study. All participants were TMS-naïve.

### Procedure

This study utilized a randomized, placebo-controlled, repeated-measures single-blind design. Each participant attended for 2 sessions which were held at least 72 h apart (see Fig. 1). Sessions were pseudorandomized and counterbalanced across participants and involved the provision of either sham or active iTBS followed by a working memory assessment at 0, 20, and 40 min post stimulation concurrent with electroencephalographic (EEG) recording. Working memory performance was assessed using the 2- and 3-back tasks.

Recordings were performed in a darkened and sound attenuated room. A 21 Ag/AgCl electrode EasyCap EEG array was applied (EasyCap, Woerthsee-Etterschlag, Germany), with CPz used as the online reference and FPz as the ground (see Fig. 1). Impedances of <5 kΩ were achieved. EEG was sampled at 1000 Hz (bandpass 0.1–100 Hz, 24 dB/octave roll-off) using a SynAmps 2 amplifier (Compumedics, Melbourne, Australia). Resting motor threshold (RMT) was then determined and iTBS subsequently applied to the left DLPFC (described below). Participants then undertook the 2- and 3-back working memory tasks immediately following stimulation, 20 and 40 min post stimulation.

### Theta Burst Stimulation

TBS was applied using a MagVenture R30/X100 (Copenhagen, Denmark) stimulator and a 70-mm diameter figure-of-8 coil. RMT was defined as the intensity required to elicit 3 of 5 consecutive motor evoked potentials with a peak-to-peak amplitude of 50 µV. TBS was then applied using an intermittent protocol (i.e., iTBS) to the left DLPFC at 80% of RMT. iTBS administration was based on Huang et al.'s (2005) protocol, where a 2 s train was repeated every 10 s for a total of 190 s. In every 2 s train, 3 pulses of stimulation were given at 50 Hz, repeated every 200 ms (600 pulses in total). The left DLPFC stimulation site was determined using the 10–20 international system for electrode placement, and was localized to F3 (Fitzgerald et al. 2009). For sham iTBS, the coil was rotated 90° about the axis of the handle.

### Working Memory Task: *n*-Back

Participants completed the working memory assessment at 0, 20, and 40 min post stimulation. Each working memory assessment was 10 min in duration and consisted of 5 min of the 2-back and 5 min of the 3-back. For the remaining 10-min period in-between assessments participants did not engage in any cognitive activity. For the *n*-back tasks, a (pseudo-) random series of the letters A to J were presented consecutively and participants were required to respond with a button press when the presented letter was the same as the letter presented either 2 or 3 trials earlier. The *n*-back task as a whole consisted of 260 trials containing 25% targets. Each letter was presented for 500 ms with a 1500-ms delay between stimuli presentations. Over the 2 sessions, each participant undertook a total of 6 blocks of the 2-back and 6 blocks of the 3-back. Alternate stimuli were used for each of these blocks.

### Behavioral Data Statistical Analysis

Data from 19 participants were analyzed. We used 2 a priori-dependent variables to assess working memory performance,

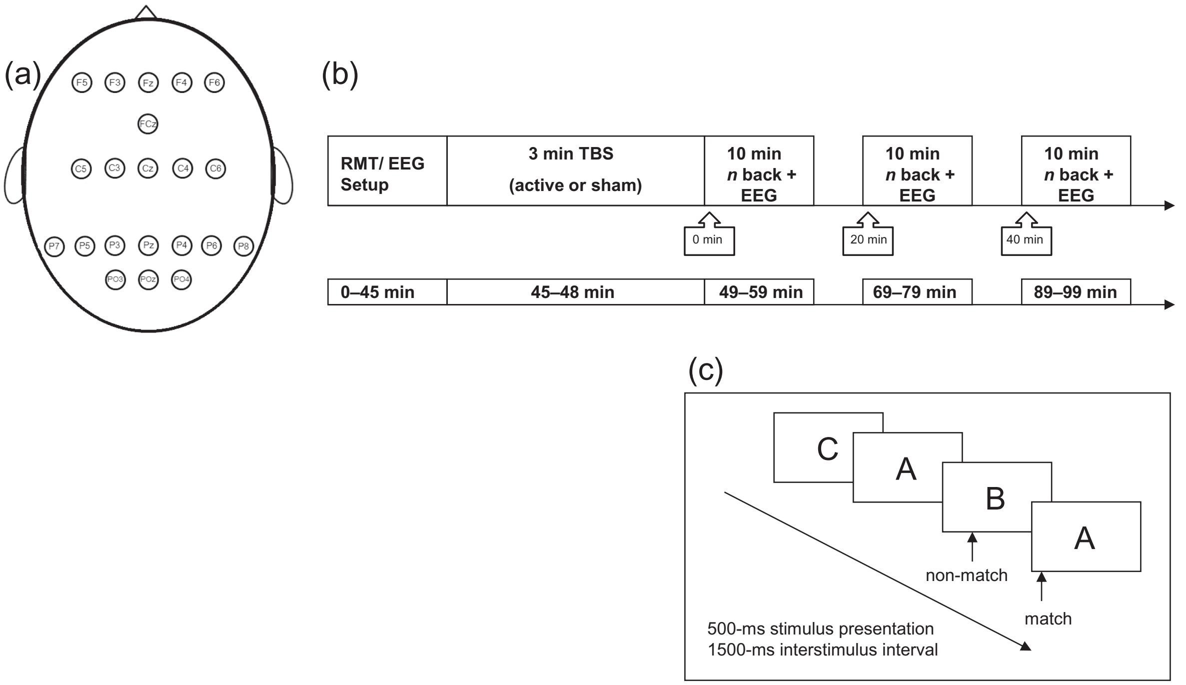


**Figure 1.** Illustration of experimental setup and protocol. (*a*) 21 EEG single electrode array (*b*) All participants underwent both 2 experimental sessions, which were spaced at least 72 h apart. Either active or sham TBS was applied for approximately 3 min. Working memory assessments occurred post stimulation at 3 time points (i.e., 0, 20, and 40 min) and consisted of the 2- and 3-back with concurrent EEG recording. (*c*) illustration of *n*-back task, depicting the 2-back condition.

**Table 2** Number of accepted epochs per condition

| | 2-back | | | | | | 3-back | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 min | | 20 min | | 40 min | | 0 min | | 20 min | | 40 min | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Active TBS | 77.63 | 13.63 | 77.84 | 12.90 | 77.21 | 12.20 | 47.95 | 8.89 | 46.68 | 6.40 | 47.42 | 6.04 |
| Sham TBS | 72.47 | 15.91 | 77.37 | 9.73 | 74.79 | 8.16 | 47.79 | 7.60 | 48.74 | 8.16 | 46.89 | 6.80 |

namely $d'$ and accurate reaction time. $d'$ is a discriminability index which takes into account the ability to correctly identify targets and to minimize false alarms and has been shown to have high sensitivity (Haatveit et al. 2010). Accurate reaction time refers to the mean reaction time in milliseconds for hits only where responses occurred prior to onset of the next stimulus. To investigate "the ability of active iTBS to enhance working memory performance over time," we undertook analysis of variances (ANOVAs) (SPSS 20.0) for $d'$ and accurate reaction time with condition (active and sham), time (0, 20, 40 min), and load (2-back, 3-back) as within-subjects factors. Post hoc analysis of simple main effects was done using pairwise comparisons, while significant interactions were further explored using one-way ANOVAs and paired-sample tests where appropriate. We also conducted a series of correlations to investigate any relationship between significant findings in the EEG and behavioral measures, which were expressed as percentage change from sham. All results were assessed using an $\alpha$ significance level of <0.05, while <0.08 was considered a trend-level finding. Outliers were detected using the outlier labeling method and were winsorized, and this was required for only 0.4% of data which is considered to be well within acceptable limits (i.e., whereby $P$ values are not affected) (Hoaglin and Iglewicz 1987).

## Electrophysiological Data Preprocessing

Data were analyzed offline in MATLAB (The Mathworks, Natick, MA, USA) using EEGLAB for preprocessing (sccn.ucsd.edu/eeglab) (Delorme and Makeig 2004), and fieldtrip for frequency and connectivity analysis (http://www.ru.nl/donders/fieldtrip) (Oostenveld et al. 2011). Data were re-referenced to averaged mastoids. Second-order Butterworth filtering was applied to the data with a bandpass from 1 to 80 Hz and also a band stop filter from 45 to 55 Hz. Data were then epoched from −0.5 to 2 s around stimulus onset for each trial, with only correctly encoded trials selected to be analyzed (encoding trials where the subsequent probe trial showed the correct response). Trials containing a response in the epoch were excluded to avoid confounds presented by motor preparation. Epochs were baseline corrected to the prestimulus period. Data were visually inspected, and epochs containing muscle artifact or excessive noise were excluded. Data were also subjected to automatic artifact rejection based on probability deviations of more than 3 SD, and based on frequencies exceeding −100 to 25 dB in the 25- to 45-Hz window in order to exclude muscle artifact. Each participant provided a minimum of 25 accepted epochs for each condition; Table 2 shows accepted epoch numbers per condition. No significant differences were detected between sham and active conditions in number of

**Table 3** Means and standard deviations of *d*′ and accurate reaction time for the 2- and 3-back post stimulation

| | 2-back | | | | | | 3-back | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 min | | 20 min | | 40 min | | 0 min | | 20 min | | 40 min | |
| | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| *d*′ | | | | | | | | | | | | |
| Active TBS | 3.24 | 0.77 | 3.68 | 0.98 | 3.55 | 0.88 | 2.45 | 0.78 | 2.47 | 0.69 | 2.39 | 0.77 |
| Sham TBS | 3.06 | 0.72 | 3.13 | 0.79 | 2.97 | 0.78 | 2.10 | 0.61 | 2.23 | 0.47 | 2.24 | 0.84 |
| Accurate reaction time | | | | | | | | | | | | |
| Active TBS | 527.18 | 151.42 | 478.17 | 86.22 | 481.67 | 60.20 | 580.35 | 157.71 | 575.21 | 140.06 | 549.95 | 137.01 |
| Sham TBS | 491.26 | 90.42 | 513.34 | 90.19 | 521.33 | 122.51 | 564.53 | 154.68 | 572.86 | 152.41 | 581.48 | 150.92 |

accepted epochs ($P < 0.10$). Eye movements and remaining muscle activity artifacts were manually selected and corrected for using a binary independent component analysis algorithm (Amari et al. 1996) using the extended option to extract sub-Gaussian sources (Lee et al. 1999).

### Frequency Domain Analysis

In order to measure power at each electrode, epochs were submitted to Mortlet wavelet decompositions (3.5 oscillation cycles with steps of 1 Hz between 4 and 8 Hz for theta and between 30 and 45 Hz for gamma). Power was then baseline corrected to the prestimulus period, and averaged over trials for each participant, condition, time, and memory load. Oscillations in the averaged theta and gamma bands were then compared between the sham and active conditions at each time point and memory load separately, making comparisons across space (averaged across time from 500 to 1350 ms and frequency from 4 to 8 Hz and from 30 to 45 Hz) using nonparametric cluster-based permutation statistics controlling for multiple comparisons. If these comparisons were significant, secondary comparisons were examined across time separately. Clusters were defined by one or more neighboring electrode with *t*-statistics at a given time point exceeding a threshold of $P < 0.05$ (dependent *t*-test). These significant electrode time points were then used for cluster-based permutation analyses, with Monte Carlo *P* values calculated from 2000 randomizations, using an $\alpha$ of $P < 0.05$. For the correlational analyses, the average power from the electrodes in the significant clusters was generated.

### Connectivity Computation

In order to measure connectivity between electrodes, epochs were submitted to a Hanning single taper time–frequency transform to determine instantaneous phase values for the complex Fourier spectra from 4 to 45 Hz with a 1-Hz resolution across sliding time windows corresponding to 3 oscillation cycles in length. The debiased estimator of the weighted phase-lagged index (wPLI) was then calculated between each electrode pair. The wPLI is a conservative measure of phase synchronization between electrodes (Vinck et al. 2011). It has the advantage of being robust against the effects of volume conduction and activity from a common reference because phase lags between sensors of near zero contribute minimally to the wPLI measure, preventing the detection of false-positive connectivity due to these artifacts. The wPLI has good test–retest reliability (Hardmeier et al. 2014). wPLI values for each participant were averaged in the frequency domain into the theta (4–8 Hz) and gamma bands (30–45 Hz), and averaged in the time domain from 650 to 900 ms following the stimulus (a time period where frequency domain analyses were found to be significant). wPLI measures of connectivity from F3 to P3 and F3 to P4 were then compared with repeated-measures ANOVAs (SPSS 20.0) for the 2- and 3-back separately with condition (active and sham), time (0, 20, 40 min), and electrode pair (F3–P3, F3–P4) as within-subjects factors.

## Results

### Behavioral Data

Order effect analysis confirmed the effectiveness of the counterbalancing of active and sham sessions, with no significant session order effects seen in either the 2- or 3-back for *d*′ (2-back: $F_{1,18} = 0.474$, $P = 0.500$; 3-back: $F_{1,18} = 0.010$, $P = 0.923$) or accurate reaction time (2-back: $F_{1,18} = 1.444$, $P = 0.245$; 3-back: $F_{1,18} = 0.089$, $P = 0.769$).

#### *d*′

Means and standard deviations for *d*′, 2-back, and 3-back are provided in Table 3, hits and false alarm rates are included in the supplementary material.

There was a significant main effect of stimulation condition ($F_{1,18} = 10.581$, $P = 0.004$), with better overall performance following active iTBS compared with sham iTBS. There was also a significant three-way interaction between stimulation condition, time and load ($F_{1,18} = 3.719$, $P = 0.034$). The effect of stimulation and time as a function of load was subsequently investigated using post hoc ANOVAs.

#### *2-Back*

*Overall effect of stimulation on performance*. There was a significant main effect of condition ($F_{1,18} = 15.136$, $P = 0.001$; $d = 1.32$), with better performance overall following active iTBS compared with sham iTBS. The condition by time interaction did not reach significance ($F_{2,36} = 2.399$, $P = 0.105$) (see Fig. 2).

*Effect of stimulation on performance over time*. Further investigation of the effect of active iTBS on WM over time, in line with the a priori hypothesis, revealed significant improvements in performance ($F_{2,36} = 5.855$, $P = 0.006$). Pairwise comparisons showed a significant improvement in performance from 0 to 20 min post stimulation (mean difference = 0.435, $P = 0.003$) that was maintained at 40 min post (0–40 min: mean difference = 0.310, $P = 0.028$; 20–40 min: mean difference = 0.124, $P = 0.376$). There was no significant change in performance over time following sham stimulation ($F_{2,36} = 0.596$, $P = 0.556$).

In addition to testing the effect of each condition on 2-back performance over time, the effect of condition at each time

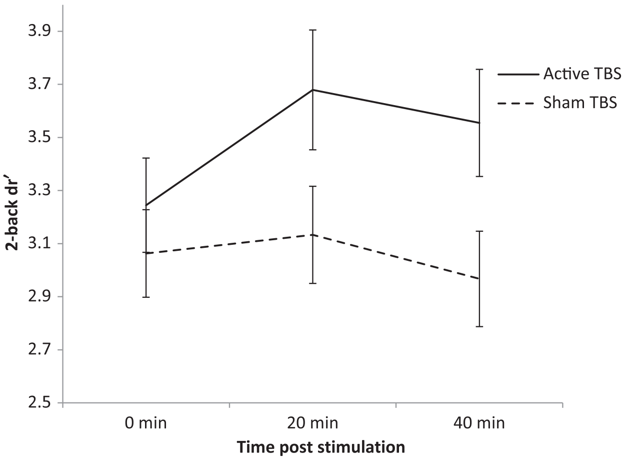


**Figure 2.** Means and standard errors of 2-back WM performance across the 3 post stimulation time points as a function of stimulation condition as assessed by $d'$.

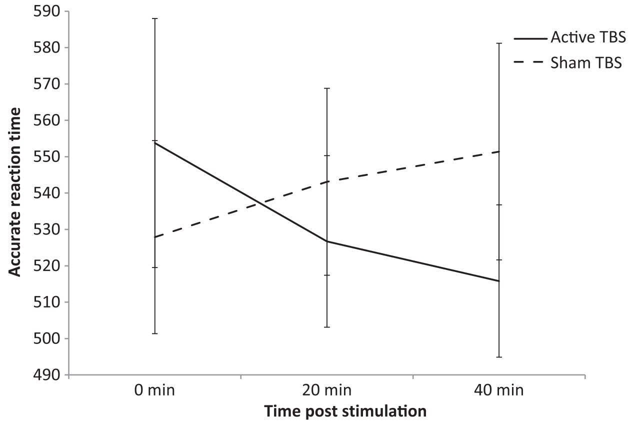


**Figure 3.** Means and standard errors of accurate reaction time for WM assessment across the 3 post stimulation time points as a function of stimulation condition.

point was also examined. While there was no effect of condition immediately following stimulation ($t_{(18)} = 1.536$, $P = 0.142$), participants exhibited significantly better performance in the active iTBS condition at both 20 ($t_{(18)} = 2.729$, $P = 0.014$; $d = 1.39$) and 40 min ($t_{(18)} = 3.661$, $P = 0.002$; $d = 1.56$) post stimulation.

#### *3-Back*

*Overall effect of stimulation on performance*. For the higher WM load, there was no main effect of condition ($F_{1,18} = 3.073$, $P = 0.097$) or condition by time ($F_{2,36} = 0.775$, $P = 0.468$).

*Effect of stimulation on performance over time*. There was no significant change in performance over time following either active iTBS ($F_{2,36} = 0.178$, $P = 0.838$) or sham stimulation ($F_{2,36} = 1.085$, $P = 0.349$).

#### *Accurate Reaction Time*

Means and standard deviations for accurate reaction time, 2-back, and 3-back are provided in Table 3.

There was no significant main effect of stimulation ($F_{1,18} = 2.389$ $P = 0.106$). A significant interaction between stimulation condition and time was found for accurate reaction time however ($F_{1,18} = 4.804$, $P = 0.014$).

Post hoc analysis revealed a trend-level improvement following active iTBS ($F_{2,36} = 3.086$, $P = 0.058$). Pairwise comparisons showed no change from 0 to 20 min (mean difference = −27.076, $P = 0.116$) or from 20 to 40 min (mean difference = −10.897, $P = 0.187$), there was however a trend-level improvement from 0 to 40 min (mean difference = −37.955, $P = 0.078$). There was no change in the sham iTBS condition ($F_{2,36} = 2.389$, $P = 0.106$). See Figure 3.

### EEG Data

#### *2-Back*

*Band power*. No significant differences were present in the theta band. Significantly more gamma power was found in active compared with sham at 0 min ($P < 0.01$), with maxima over the left parietal region ($P = 0.005$) and right parietal region ($P = 0.006$) (see Fig. 4*a*). The analysis across time showed right parietal cluster significance from 660 to 715 ms following the stimuli ($P = 0.009$), but the left parietal cluster did not survive controls for multiple correction (see Fig. 4*b*). This increased gamma power following active compared with sham persisted from 0 to 20 min, with maximum power in the right parietal region ($P = 0.008$) (see Fig. 5*a*). Analysis across time showed significance from 770 to 890 ms following the stimuli ($P = 0.001$) (see Fig. 5*b*).

*Connectivity*. A significant main effect of condition was found in the theta band ($F_{1,18} = 6.441$, $P = 0.021$), with greater theta wPLI from F3 to P3 and P4 following active stimulation compared with sham. No other main effects or interactions were found (all $P > 0.05$).

#### *3-Back*

*Band power*. No differences were present in the theta band. There was significantly more gamma power found in active compared with sham at 0 min ($P < 0.01$), again with maxima over the left parietal region ($P = 0.004$) and right parietal region ($P = 0.015$) (see Fig. 6*a*). The analysis across time showed right parietal cluster significance from 550 to 580 ms following the stimuli ($P = 0.042$), but the left parietal cluster did not survive controls for multiple correction (see Fig. 6*b*).

*Connectivity*. There were no significant differences in wPLI estimates of connectivity for either theta or gamma in the 3-back.

### Correlations

Correlations between the behavioral and EEG changes did not reach significance. There were near significant negative correlations between 2-back accurate reaction time and theta connectivity from F3 to P3 at 20 min ($\rho = -0.407$, $P = 0.084$) and 40 min ($\rho = -0.453$, $P = 0.052$); whereby increased connectivity was associated with decreased reaction time following active stimulation.

### Tolerability of iTBS

All participants tolerated iTBS well with no adverse events reported.

## Discussion

Significant and large improvements in working memory performance were seen following active iTBS, with effect sizes ranging from $d = 1.39$ to 1.56. Task-related EEG enhancements were also seen, with significantly increased theta connectivity between frontal and parietal regions, and greater parietal gamma

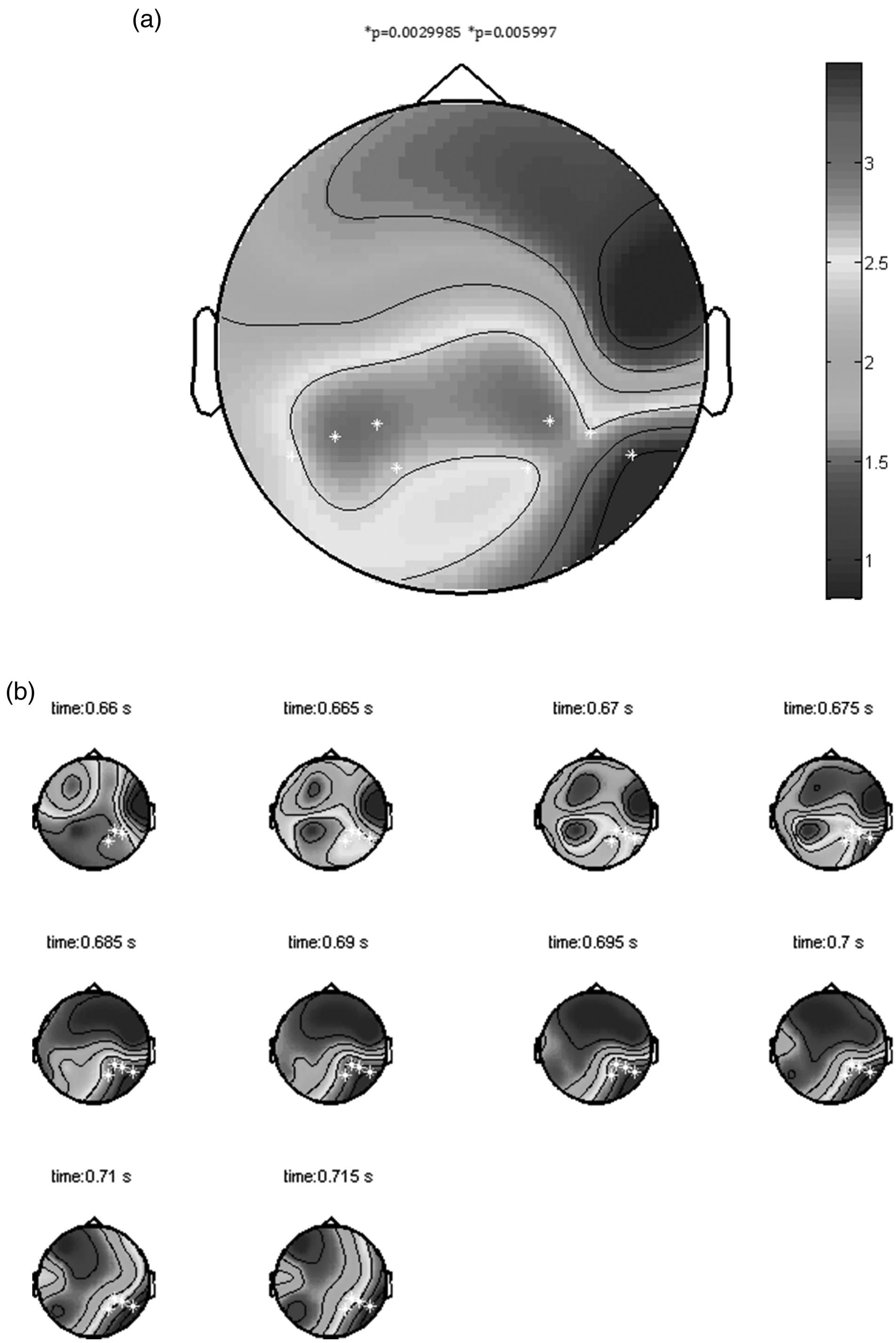


**Figure 4.** Gamma power during 2-back encoding at T0 following active iTBS when compared with sham iTBS. (*a*) Significant increases in gamma power in left and right PPC during the averaged window of interest 500–1350 ms. (*b*) Significant increase in gamma power measured across time window lasted from 660 to 715 ms in right PPC. The color bar represents *t*-values.

power, following active stimulation compared with sham. More specifically, there was no effect of condition on 2-back accuracy immediately following stimulation (i.e., 0 min) however a significant improvement following active iTBS was evident by 20 min post stimulation and maintained at 40 min. Active iTBS resulted in significantly greater overall theta connectivity from the stimulated left DLPFC to right and left parietal regions during performance of the 2-back. There was also significantly greater task-related gamma power in both left and right parietal regions both immediately following active stimulation (i.e., 0 min) and at 20 min post stimulation for the 2-back. There was no significant improvement in 3-back accuracy, there was however greater gamma power over left and right parietal regions during the 3-back immediately following active stimulation compared

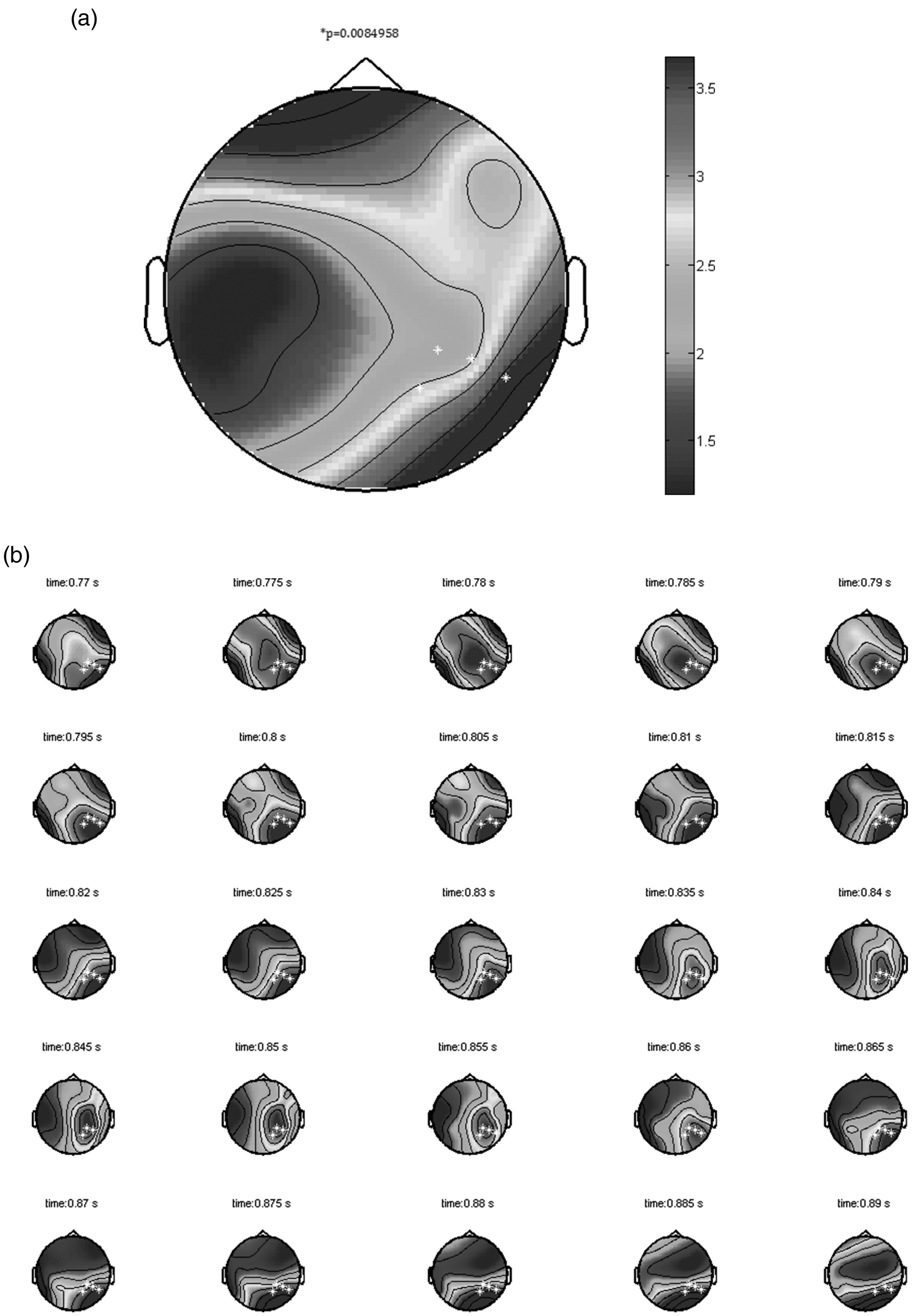


**Figure 5.** Gamma power during 2-back encoding at T1 following active iTBS when compared with sham iTBS. (*a*) Significant increases in gamma power in right PPC during the averaged window of interest 500–1350 ms. (*b*) Significant increase in gamma power across time window lasted from 770 to 890 ms. The color bar represents *t*-values.

with sham. With respect to accurate reaction time, there was a trend-level improvement over time following active iTBS only, this was irrespective of working memory load.

The lack of a significant behavioral improvement in 3-back accuracy, even in the presence of enhanced gamma power, is not necessarily unexpected and is consistent with our previous work suggesting a possible behavioral "enhancement ceiling effect" during 3-back performance in healthy controls (Hoy et al. 2013). Namely that despite iTBS significantly enhancing task-related gamma power during the 3-back, the fact that the behavioral

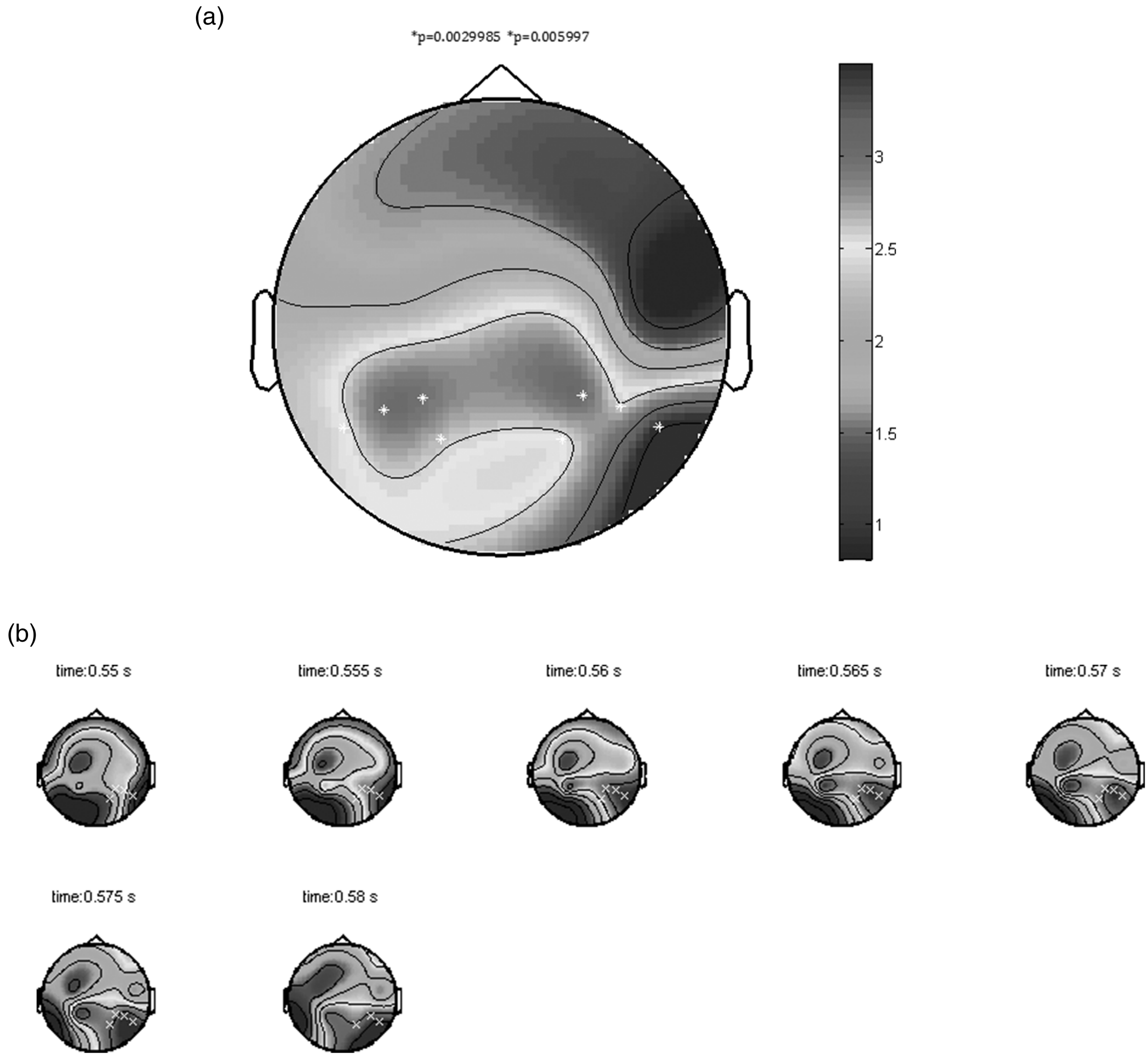


**Figure 6.** Gamma power during 3-back encoding at T0 following active iTBS when compared with sham iTBS. (*a*) Significant increases in gamma power in left and right PPC during the averaged window of interest 500 to 1350 ms. (*b*) Significant increase in gamma power across time window lasted from 550 to 580 ms in right PPC. The color bar represents *t*-values.

improvement did not reach significance [active (M ± standard error (SE)] = 2.434 ± 0.152, sham (M ± SE) = 2.188 ± 1.38; P = 0.097) is consistent with participants already performing at or close to ceiling. Suggesting that even significant improvements in cognitive processing cannot alter the performance ceiling and that, at least in healthy controls, the degree of stimulation related enhancement is limited to within an individual's own innate potential.

The improvement in performance of the 2-back in the current study is markedly more robust than that shown by previous research into the effects of noninvasive brain stimulation on cognitive enhancement. Recent meta-analyses have reported only small effect sizes of HF-TMS and a-tDCS on working memory performance across both patients and controls ($g = 0.14$–$0.254$), with healthy controls showing the smallest effects (Brunoni and Vanderhasselt 2014). There has been very limited investigation of alternative approaches such as iTBS however. While there have been a number of studies of "online" TBS that have investigated how stimulation during a task affects outcomes (Cazzoli et al. 2009; Rounis et al. 2010; Verbruggen et al. 2010; Morgan et al. 2013), there has been only one study to date that has looked at iTBS to the DLPFC in an "offline" paradigm to assess its impact on post-stimulation cognitive performance (Grossheinrich et al. 2009). That study looked at the effect of left DLPFC iTBS on post-stimulation working memory performance, along with a number of variables, finding no significant effects of iTBS on working memory performance. There were however a number of methodological differences with our study (Grossheinrich et al. 2009). Three forms of TBS (iTBS, continuous-TBS and sham TBS) were performed in a single day with only an hour wash out between sessions, working memory was assessed using pen and paper tasks which lack the sensitivity of computerized cognitive assessment, and performance was only assessed at one time point following stimulation (Grossheinrich et al. 2009). Therefore, our divergent positive findings could be due to a number of factors including a longer washout period (72 h), more sensitive measures of performance, and the conduct of multiple assessments post stimulation especially as it was only at 20 min following iTBS that we saw a significant improvement in performance compared with sham. The strong effects seen in the current study could be explained by the greater convergence between the posited mechanisms of action of iTBS and the neurophysiological processes important for cognitive function broadly and possibly even working memory specifically.

Our findings could be the result of iTBS priming the optimal cortical environment for cognitive performance in general, with effects seen in working memory simply due to the nature of the task employed in the study. Cortical activity is dependent on the balance of excitatory (*E*) to inhibitory (*I*) synaptic inputs and studies have shown that there appears to be an optimal *E*/*I* ratio for maximal information capacity and information transmission (Beggs and Plenz 2003; Shew et al. 2011; Yizhar et al. 2011). It has been theorized that noninvasive brain stimulation techniques induce improvements via modulation of this *E*/*I* balance (Krause et al. 2013). This conceptualization goes some way to explain the broadness of cognitive effects reported with these techniques, whereby stimulation produces a more optimal *E*/*I* balance which will act to enhance cognitive performance of whichever task is then undertaken given it has some relevance to the brain region being stimulated. It may also help to account for the marked interindividual differences seen with these techniques as enhancement of the *E*/*I* balance, and hence performance, is likely to only occur within that individual's own range of innate ability.

Compared with the standard forms of stimulation, iTBS more closely mimics the brain's natural neuronal firing patterns and has been shown to produce more robust changes in cortical excitability and subsequently enhanced neural firing and synchrony (Huang et al. 2005; Benali et al. 2011). Neural synchrony refers to the coordinated firing of connected brain regions, and is considered essential for the integration of neural networks and cognitive performance (Klimesch 1996). In the current study, iTBS may have resulted in modulation of the *E*/*I* balance to a more optimal ratio and thus primed the system for greater task-related neuronal synchrony and behavioral enhancement. Such a theory would be consistent with the time course of findings, namely that while the neurophysiological enhancement occurred immediately, and persisted, the behavioral improvements were first apparent at the 20-min time point. This pattern of results supports a possible priming effect, whereby the initial neurophysiological effects of iTBS provide an optimized cortical environment for improved performance at subsequent time points, allowing participants to obtain greater gains with repeated performance of the task.

A possible alternative explanation for the current findings is that iTBS produced such large effects on working memory performance due to enhancement of cortical activity specific to working memory. TMS has been shown to entrain cortical oscillations at its stimulation frequency (Thut et al. 2011) and iTBS stimulation provides gamma frequency stimulation (50 Hz) at theta frequency intervals; both of which have been strongly implicated in working memory functioning (Lisman 2010). In the current study following active iTBS, compared with sham, theta and gamma neural synchronization in particular were significantly enhanced. Specifically, there was significantly greater theta coherence between the site of stimulation (left DLPFC) and left and right parietal cortices during the 2-back compared with sham iTBS. Theta oscillations play a critical role in integration of the different brain regions required for working memory, with synchronous theta activity between prefrontal and posterior parietal regions associated with successful encoding in working memory (Sederberg et al. 2003; Sauseng et al. 2010). Functionally, this synchronization is thought to play a role in the integration of posterior association cortex (where sensory information is stored) and prefrontal cortex (where relevant current information is held and continuously updated) (Von Stein and Sarnthein 2000). Following iTBS there was also increased 2-back task-related gamma power over the left and right parietal regions immediately following stimulation and in the right parietal cortex 20 min later. Locally driven gamma activity is thought to relate to information binding reflecting ongoing neural computation, whereby increases in gamma are thought to reflect the enhanced sensory processing, with right PPC particularly relevant for the encoding of character strings as is the case in the *n*-back (Sarnthein et al. 1998). The neurophysiological effects of iTBS stimulation on task-related EEG activity seen in the current study would be consistent with enhancement of specific neurophysiological processes underlying working memory.

The current findings are promising that they show a more substantial effect of iTBS compared with the more standard forms of noninvasive brain stimulation investigated to date. However, while the effect sizes are large, it remains unclear how significantly these improvements would impact "real world" cognitive function in healthy controls. For example, the lack of change in the more cognitively demanding 3-back could be interpreted as lending weight to the theory that the degree of cognitive enhancement possible in the healthy brain is indeed limited (Hoy et al. 2013). Importantly, there is research to suggest that this is not the case in patients with impaired cognitive functioning (Hoy et al. 2014). It is likely that these techniques will have considerably more relevance to the restoration of impaired brain function underlying cognitive symptoms of illnesses such as schizophrenia, dementia, Parkinson's disease, and even head injury. For example, there is strong evidence for the role of impaired *E*/*I* balance, and impaired neural synchrony, in the cognitive symptoms of schizophrenia, and as such the results of the current study would indicate that iTBS may be particularly suited to the treatment of these deficits (Yizhar et al. 2011). There remains considerable work to be done prior to such use, however.

The current results require replication, preferably in a larger sample as well as extension into patient populations. While the current sample size could be considered moderate, the large effect sizes do indicate adequate power. In addition, future research to address the limitations of the current study with respect to localization and sham methodology is needed, (i.e., using neuronavigation to more accurately localize DLPFC and utilization of a sham coil to eliminate the impact of potential stimulation with the "coil rotated 90°" method). Also, as the current study was specifically interested in the ability of iTBS to induce a cortical state that would enhance post-stimulation cognitive performance, we did not utilize a prestimulation baseline instead comparing post-stimulation performances between active and sham stimulation. Future research investigating the more general effect of iTBS on cognitive performance should utilize a prestimulation baseline. In addition, research into the mechanisms of action of iTBS induced cognitive enhancement, namely whether modulation of the *E*/*I* balance or entrainment of oscillations does occur following stimulation, is required using techniques such as combined TMS-EEG. More detailed investigation of the duration of effects is also needed, while our results showed significantly improved performance out to the final time point of 40 min, it remains unclear how longer the effects would last. In addition, investigation of the degree and duration of effects following repeated sessions of stimulation is needed.

## Conclusions

In conclusion, the current study indicates that a single session of iTBS lasting approximately 3 min is able to significantly improve working memory performance in healthy controls, seemingly via enhancement of relevant neurophysiological processes. These findings provide support for the use of more specialized stimulation approaches in neuromodulation in order to enhance effectiveness of these techniques.

## Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

## Funding

K.H., B.F., N.R., and P.F. are supported by National Health and Medical Research Council (NHMRC) fellowships. This research was supported by a Monash University grant.

## Notes

*Conflict of Interest:* P.F. has received equipment for research from Brainsway Ltd, Medtronic Ltd, and MagVenture A/S, and funding for research from Cervel Neurotech and Neuronetics Ltd. P.F. has received consultancy fees as a scientific advisor for Bionomics.

## References

Amari S, Cichocki A, Yang H. 1996. A new learning algorithm for blind signal separation. Adv Neural Inf Process Syst. 8:757–763.

Baddeley A. 2003. Working memory: looking back and looking forward. Nat Rev Neurosci. 4(10):829–839.

Beggs JM, Plenz D. 2003. Neuronal avalanches in neocortical circuits. J Neurosci. 23(35):11167–11177.

Benali A, Trippe J, Weiler E, Mix A, Petrasch-Parwez E, Girzalsky W, Eysel UT, Erdmann R, Funke K. 2011. Theta-burst transcranial magnetic stimulation alters cortical inhibition. J Neurosci. 31(4):1193–1203.

Brunoni AR, Vanderhasselt MA. 2014. Working memory improvement with non-invasive brain stimulation of the dorsolateral prefrontal cortex: a systematic review and meta-analysis. Brain Cogn. 86:1–9.

Cazzoli D, Wurtz P, Müri RM, Hess CW, Nyffeler T. 2009. Interhemispheric balance of overt attention: a theta burst stimulation study. Eur J Neurosci. 29(6):1271–1276.

Coffman BA, Clark VP, Parasuraman R. 2014. Battery powered thought: enhancement of attention, learning, and memory in healthy adults using transcranial direct current stimulation. Neuroimage. 85:895–908.

Delorme A, Makeig S. 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods. 134(1):9–21.

Di Lazzaro V, Pilato F, Dileone M, Profice P, Oliviero A, Mazzone P, Insola A, Ranieri F, Meglio M, Tonali PA, et al. 2008. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. J Physiol. 586 (16):3871–3879.

Fitzgerald PB, Fountain S, Daskalakis ZJ. 2006. A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. Clin Neurophysiol. 117(12):2584–2596.

Fitzgerald PB, Maller JJ, Hoy KE, Thomson R, Daskalakis ZJ. 2009. Exploring the optimal site for the localization of dorsolateral prefrontal cortex in brain stimulation experiments. Brain Stimul. 2(4):234–237.

Grossheinrich N, Rau A, Pogarell O, Hennig-Fast K, Reinl M, Karch S, Dieler A, Leicht G, Mulert C, Sterr A, et al. 2009. Theta burst stimulation of the prefrontal cortex: safety and impact on cognition, mood, and resting electroencephalogram. Biol Psychiatry. 65(9):778–784.

Haatveit BC, Sundet K, Hugdahl K, Ueland T, Melle I, Andreassen OA. 2010. The validity of d prime as a working memory index: results from the “Bergen n-back task”. J Clin Exp Neuropsychol. 32(8):871–880.

Hardmeier M, Hatz F, Bousleiman H, Schindler C, Stam CJ, Fuhr P. 2014. Reproducibility of functional connectivity and graph measures based on the phase lag index (PLI) and weighted phase lag index (wPLI) derived from high resolution EEG. PLoS ONE. 9(10):e108648.

Hauser TU, Rotzer S, Grabner RH, Mérillat S, Jäncke L. 2013. Enhancing performance in numerical magnitude processing and mental arithmetic using transcranial Direct Current Stimulation (tDCS). Front Hum Neurosci. 7:244. doi: 10.3389/fnhum.2013.00244.

Hecht D, Walsh V, Lavidor M. 2010. Transcranial direct current stimulation facilitates decision making in a probabilistic guessing task. J Neurosci. 30(12):4241–4245.

Hoaglin DC, Iglewicz B. 1987. Fine-tuning some resistant rules for outlier labeling. J Am Stat Assoc. 82(400):1147–1149.

Hoy KE, Arnold SL, Emonson MR, Daskalakis ZJ, Fitzgerald PB. 2014. An investigation into the effects of tDCS dose on cognitive performance over time in patients with schizophrenia. Schizophr Res. 155(1):96–100.

Hoy KE, Emonson MR, Arnold SL, Thomson RH, Daskalakis ZJ, Fitzgerald PB. 2013. Testing the limits: investigating the effect of tDCS dose on working memory enhancement in healthy controls. Neuropsychologia. 51(9):1777–1784.

Hrabetova S, Serrano P, Blace N, Tse HW, Skifter DA, Jane DE, Monaghan DT, Sacktor TC. 2000. Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. J Neurosci. 20 (12):81–6.

Huang YZ, Chen RS, Rothwell JC, Wen HY. 2007. The after-effect of human theta burst stimulation is NMDA receptor dependent. Clin Neurophysiol. 118(5):1028–1032.

Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC. 2005. Theta burst stimulation of the human motor cortex. Neuron. 45(2):201–206.

Klimesch W. 1996. Memory processes, brain oscillations and EEG synchronization. Int J Psychophysiol. 24(1):61–100.

Krause B, Márquez-Ruiz J, Kadosh RC. 2013. The effect of transcranial direct current stimulation: a role for cortical excitation/inhibition balance? Front Hum Neurosci. 7:602. doi: 10.3389/fnhum.2013.00602.

Lee T, Girolami M, Sejnowski T. 1999. Independent component analysis using an extended infomax algorithm for mixed sub-Gaussian and super-Gaussian sources. Neural Comput. 11:417–441.

Lisman J. 2010. Working memory: the importance of theta and gamma oscillations. Curr Biol. 20(11):R490–R492.

Luber B, Lisanby SH. 2014. Enhancement of human cognitive performance using transcranial magnetic stimulation (TMS). Neuroimage. 85:961–970.

Morgan HM, Jackson MC, van Koningsbruggen MG, Shapiro KL, Linden DE. 2013. Frontal and parietal theta burst TMS impairs working memory for visual-spatial conjunctions. Brain Stimul. 6(2):122–129.

Oberman L, Edwards D, Eldaief M, Pascual-Leone A. 2011. Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. J Clin Neurophysiol. 28(1):67.

Oostenveld R, Fries P, Maris E, Schoffelen J. 2011. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. Comput Intell Neurosci. 9. http://dx.doi.org/10.1155/2011/156869.

Rossi S, Rossini PM. 2004. TMS in cognitive plasticity and the potential for rehabilitation. Trends Cogn Sci. 8(6):273–279.

Rounis E, Maniscalco B, Rothwell JC, Passingham RE, Lau H. 2010. Theta-burst transcranial magnetic stimulation to the prefrontal cortex impairs metacognitive visual awareness. Cogn Neurosci. 1(3):165–175.

Sarnthein J, Petsche H, Rappelsberger P, Shaw GL, Von Stein A. 1998. Synchronization between prefrontal and posterior association cortex during human working memory. PNAS. 95 (12):7092–7096.

Sauseng P, Griesmayr B, Freunberger R, Klimesch W. 2010. Control mechanisms in working memory: a possible function of EEG theta oscillations. Neurosci Biobehav Rev. 34(7):1015–1022.

Sederberg PB, Kahana MJ, Howard MW, Donner EJ, Madsen JR. 2003. Theta and gamma oscillations during encoding predict subsequent recall. J Neurosci. 23(34):10809–10814.

Sheehan DV, Lecrubier Y, Sheehan KH, Janavs J, Weiller E, Keskiner A, Schinka J, Knapp E, Sheehan MF, Dunbar GC. 1997. The validity of the Mini International Neuropsychiatric Interview (MINI) according to the SCID-P and its reliability. Eur Psychiatry. 12(5):232–241.

Shew WL, Yang H, Yu S, Roy R, Plenz D. 2011. Information capacity and transmission are maximized in balanced cortical networks with neuronal avalanches. J Neurosci. 31(1):55–63.

Thut G, Veniero D, Romei V, Miniussi C, Schyns P, Gross J. 2011. Rhythmic TMS causes local entrainment of natural oscillatory signatures. Curr Biol. 21(14):1176–1185.

Verbruggen F, Aron AR, Stevens MA, Chambers CD. 2010. Theta burst stimulation dissociates attention and action updating in human inferior frontal cortex. PNAS. 107(31):13966–13971.

Vinck M, Oostenveld R, van Wingerden M, Battaglia F, Pennartz CM. 2011. An improved index of phase-synchronization for electrophysiological data in the presence of volume-conduction, noise and sample-size bias. Neuroimage. 55 (4):1548–1565.

Von Stein A, Sarnthein J. 2000. Different frequencies for different scales of cortical integration: from local gamma to long range alpha/theta synchronization. Int J Psychophysiol. 38(3): 301–313.

Yizhar O, Fenno LE, Prigge M, Schneider F, Davidson TJ, O'Shea DJ, Sohal VS, Goshen I, Finkelstein J, Paz JT, et al. 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature. 477(7363):171–178.