Received date:- 20-Oct-2014

Revised Date:- 28-Nov-2015

Accepted Date: 01-Dec-2015

Article Type: Research Report

Combined transcranial alternating current stimulation and cTBS: a novel approach for neuroplasticity induction

Mitchell R. Goldsworthy[1,*], Ann-Maree Vallence[1,2,*], Ruiting Yang[1], Julia B. Pitcher[1], Michael C. Ridding[1]

* Authors contributed equally

[1]The Robinson Research Institute, School of Medicine, University of Adelaide, Adelaide 5005, Australia.

[2]School of Psychology and Exercise Science, Murdoch University, WA, Australia.

Correspondence to Dr Mitchell Goldsworthy

Email: mitchell.goldsworthy@adelaide.edu.au

Address: NeuroPAD, Robinson Research Institute

School of Medicine

University of Adelaide SA 5005

Ph: +61 8 8313 1323

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ejn.13142

Running title: Combined tACS-cTBS

Keywords: human; theta burst stimulation; electroencephalography; alpha rhythm; entrainment

Abstract

Non-invasive brain stimulation can induce functionally relevant plasticity in the human cortex, making it potentially useful as a therapeutic tool. However, the induced changes are highly variable between individuals, potentially limiting research and clinical utility. One factor that might contribute to this variability is the level of cortical inhibition at the time of stimulation. The alpha rhythm (~8–13 Hz) recorded with electroencephalography (EEG) is thought to reflect pulsatile cortical inhibition; therefore, targeting NIBS to particular phases of the alpha rhythm may provide an approach to enhance plasticity induction. Transcranial alternating current stimulation (tACS) has been shown to entrain cortical oscillations in a frequency-specific manner. We investigated whether the neuroplastic response to continuous theta burst stimulation (cTBS) was enhanced by timing bursts of stimuli to the peak or the trough of a tACS-imposed alpha rhythm. While motor evoked potentials (MEPs) were unaffected when cTBS was applied in-phase with the peak of the tACS-imposed oscillation, MEP depression was enhanced when cTBS was applied in-phase with the trough. This enhanced MEP depression was dependent on the individual peak frequency of the endogenous alpha rhythm recorded with EEG prior to stimulation, and was strongest in those participants classified as non-responders to standard cTBS. These findings suggest that tACS may be used in combination with cTBS to enhance the plasticity response. Furthermore, the

peak frequency of endogenous alpha, as measured with EEG, may be used as a simple marker to pre-select those individuals likely to benefit from this approach.

Introduction

A number of non-invasive brain stimulation (NIBS) protocols have been developed that can induce short-lasting plasticity in the human cortex, as reflected by a change in cortical excitability. It is thought that the induced changes are brought about by mechanisms similar to the long-term potentiation (LTP) and long-term depression (LTD) described in animal studies (Hoogendam *et al.*, 2010). While NIBS can induce changes that interact with behaviour and may have therapeutic potential for several neurological disorders, its utility is currently limited by large intra- and inter-individual response variability (Ridding & Ziemann, 2010). To maximise the opportunity for the induction of behaviourally and clinically relevant change, it is important to understand and manipulate the determinants of this variability.

It is well known that the level of inhibition within the brain influences plasticity induction (Hess *et al.*, 1996; Ziemann *et al.*, 1998; Komaki *et al.*, 2007; Clarkson *et al.*, 2010; Cash *et al.*, 2014). It has been suggested that the alpha rhythm (~8–13 Hz), recorded in the human electroencephalogram (EEG), reflects phasic modulations of cortical inhibition (Jensen & Mazaheri, 2010; Mathewson *et al.*, 2011). Support for this suggestion comes from both animal and human research: pyramidal cell firing in the macaque is greater on the down-going phase of the alpha oscillation than on the up-going phase (Haegens *et al.*, 2011), and transcranial magnetic stimulation (TMS) induced phosphene detection is greater during the

trough than the peak of the alpha oscillation recorded in the human visual cortex (Dugue *et al.*, 2011). Therefore, targeting stimuli of plasticity-inducing NIBS protocols to the trough of the alpha oscillation, that is, targeting the cortex during hypothesised 'windows' of reduced inhibition, might prove a useful approach for enhancing plasticity induction.

While targeting stimuli to particular phases of the endogenous alpha oscillation is technically very challenging, an alternate approach might be to entrain brain oscillations by applying an exogenous oscillatory stimulus. Evidence suggests that transcranial alternating current stimulation (tACS) can result in specific entrainment of cortical oscillations (Pogosyan *et al.*, 2009; Helfrich *et al.*, 2014). Here, we examine whether the LTD-like plasticity response to a NIBS paradigm known as continuous theta burst stimulation (cTBS) (Huang *et al.*, 2005) could be increased by timing the stimulation to the peak or the trough of a tACS-imposed alpha oscillation.

Materials and methods

Participants

21 right-handed subjects (mean ± SEM: 22.6 ± 0.6 years; 11 males) participated in three experimental sessions. All sessions were conducted in the afternoon to minimise time-of-day influences (Sale *et al.*, 2007), and sessions were separated by ≥2 days (Goldsworthy *et al.*, 2012a; Hamada *et al.*, 2013; Vallence *et al.*, 2013). All participants provided written, informed consent and were screened for contraindications to TMS (Rossi *et al.*, 2009) prior to enrolment. The study was conducted in accordance with the Declaration of Helsinki and was approved by the University of Adelaide Human Research Ethics Committee.

Transcranial magnetic stimulation

Electromyographic (EMG) activity was recorded from the relaxed right first dorsal interosseous (FDI) muscle using surface electrodes (belly-tendon configuration). The EMG signal was amplified (×1000; CED 1902 amplifier), band pass filtered (20–1000 Hz) and digitized at a sampling rate of 5 kHz (CED 1401 interface). A Magstim-200 stimulator generated single-pulse stimuli, delivered through a figure-of-eight coil (90 mm) placed tangentially to the scalp with the handle pointing backward, 45° away from the midline. Suprathreshold pulses were delivered over the left primary motor cortex to identify the optimal site (i.e., 'hotspot') for consistently evoking MEPs in the relaxed FDI, and this site was marked on the scalp. The TMS intensity that elicited MEPs of ~1mV (SI_{1mV}) in the relaxed FDI was determined at baseline and was used to examine changes in MEP amplitude post-intervention. Blocks of 15 single-pulse TMS trials (inter-trial interval of 7 s with 10% variance) were delivered twice at baseline (averaged to produce a single baseline measure), as well as at 0, 5, 10, 20, and 30 min post-intervention (Fig. 1A).

cTBS was delivered using a Double-Cooled-Coil-System coil (70 mm, Magstim). Short bursts of three pulses were delivered at 50 Hz every 200 ms for 40 s (Huang *et al.*, 2005). Resting motor threshold (RMT) was determined prior to the plasticity protocol in each session, and was defined as the minimum intensity (as a percentage of maximal stimulator output) required to elicit MEPs in the relaxed FDI ≥50 μV in at least 5/10 consecutive trials. cTBS intensity was set to 70% of RMT (Gentner *et al.*, 2008; Goldsworthy *et al.*, 2012a; Goldsworthy *et al.*, 2014).

Transcranial alternating current stimulation

tACS was delivered using a Magstim transcranial direct current stimulator plus (Eldith DC-stimulator, Neuroconn, Germany), and was applied using 5 x 7 cm water-soaked sponges with the anode placed over the FDI hotspot and the cathode placed over the contralateral orbit. Electrode impedance was maintained below 55 kΩ. tACS was applied as a 10 Hz sinusoidal waveform with no DC offset at an intensity of 1 mA.

Electroencephalography

60 s of continuous EEG was recorded at baseline in each session using Ag/AgCl disk electrodes (10 mm diameter) arranged in a bipolar montage, with one electrode placed at the FDI hotspot (active) and one at Fz (reference). Impedance was kept below 5 kΩ. Prior to recording, all participants were instructed to relax their face and hand muscles, keep their eyes open, and look straight ahead. EEG signals were amplified (×10,000; CED 1902 amplifier), band pass filtered (0.5–100 Hz) and digitized at a sampling rate of 2 kHz (CED 1401 interface).

Procedure

As shown in Figure 1B, cTBS was applied in combination with tACS using three different protocols: 'Sham', 'Peak', and 'Trough'. For the Peak protocol, bursts of three TMS pulses (each separated by 20 ms) were delivered centred around the peak of the sinusoidal waveform, with the first pulse of the burst delivered 20 ms prior to the peak, the second pulse delivered at the peak, and the third pulse delivered 20 ms after the peak (18, 90, 162° for the first, second, and third pulses, respectively). Conversely, for the Trough protocol, bursts were

delivered centred around the trough of the sinusoidal waveform (198, 270, 342° for the first, second, and third pulses, respectively). For both Peak and Trough protocols, bursts were delivered on every second oscillation of the applied sinusoidal waveform (i.e., conventional 5 Hz cTBS protocol) for the full 40 s. For the Sham protocol, tACS was applied for 300 ms (with pulses at 108, 180, and 252°) and then switched off while cTBS continued for 40 s. All participants completed all three protocols; the order in which participants completed each protocol was pseudo-randomised (seven participants completed Sham first, eight completed Peak first, and six completed Trough first).

Data Analysis

MEP trials were excluded from analysis on the basis of background EMG activity being present during the 200-ms period preceding the TMS pulse (less than 5% of all trials). Peak-to-peak MEP amplitude (in mV) was measured for each participant, and mean amplitude was calculated for each block of trials at each time point. Mean MEP amplitudes for the two baseline recording blocks were averaged, and mean MEP amplitudes for all time points were normalised to this baseline value.

EEG data were divided into 2-s epochs. Eye blinks and other artifacts were identified by visual inspection, and artifact-contaminated epochs were removed from further analysis (on average, 20.3 ± 0.7 artifact-free epochs remained). EEG data from two subjects were excluded from analysis due to excessive artifacts. The frequency spectrum of each epoch was calculated using Fast Fourier Transform, and power in the alpha frequency band (8–13.5 Hz) was calculated for each 2-s epoch by summing the power in bins of 0.5 Hz. Mean spectral

alpha power was calculated by averaging across all artifact-free epochs, and this was normalised to the total summed power (averaged across epochs) in the frequency range 0.5–40 Hz.

Normalised MEP data were analysed using repeated-measures ANOVA (RM-ANOVA) with PROTOCOL (three levels: Sham, Peak, and Trough) and TIME (five levels: 0, 5, 10, 20, and 30 min post-intervention) as within-subject factors. Mean MEP amplitudes (normalised to baseline) were averaged across all post-intervention time points for each experimental protocol, and the tACS-cTBS response (i.e., the extent to which tACS modulated the MEP response to cTBS) was calculated for each participant by subtracting grand-averaged post-intervention MEP amplitudes for the Peak and Trough protocols from that recorded for the Sham tACS protocol. Linear regression analyses were performed to examine the relationships between baseline alpha and the tACS-cTBS response. Cook's distance was used to identify influential observations in the regression analyses (Cook, 1977), with values exceeding three times the group mean classified as statistical outliers.

Finally, participants were grouped based on their response to cTBS in the Sham tACS condition. Participants who showed an overall decrease in MEP amplitudes following Sham tACS-cTBS [i.e., grand average of all post-intervention MEP amplitudes (normalised to baseline) < 1.0] were classified as 'responders'. Conversely, participants who showed no change or an increase in MEP amplitudes [i.e., grand average of all normalised post-intervention MEP amplitudes ≥ 1.0] were classified as 'non-responders'. A mixed-design ANOVA was performed with PROTOCOL and TIME as within-subject factors, and GROUP (two levels: responders and non-responders) as the between-subject factor.

Analyses were performed with either IBM SPSS Statistics 20 (IBM SPSS, Armonk, NY, USA), SigmaPlot 12.3 (Systat Software Inc., San Jose, CA), or MATLAB R2013a (The MathWorks Inc., USA), and were two-tailed. Data were checked for normality using the Kolmogorov-Smirnov test, and Huynh-Feldt corrections were used for analyses in which the assumption of sphericity was violated (Mauchly's test of sphericity; for simplicity, uncorrected degrees of freedom are reported). Statistical significance was accepted for $P \leq 0.05$.

Results

Influence of tACS-cTBS on MEP amplitudes

There were no differences in RMT ($F_{2,40} = 0.49$, $P = 0.57$), SI_{1mV} ($F_{2,40} = 1.55$, $P = 0.23$), or baseline MEP amplitude ($F_{2,40} = 0.22$, $P = 0.81$) between experimental sessions. Overall, there was no difference in mean MEP amplitudes between post-intervention time points (TIME: $F_{4,80} = 1.23$, $P = 0.31$) and there was no PROTOCOL*TIME interaction ($F_{8,160} = 0.92$, $P = 0.51$); however, a main effect of PROTOCOL was observed ($F_{2,40} = 4.19$, $P = 0.02$). Although post-intervention MEP amplitudes did not differ between the Peak and Sham protocols ($F_{1,20} = 0.74$, $P = 0.40$), MEP depression was greater in the Trough protocol compared with both Sham ($F_{1,20} = 5.71$, $P = 0.03$) and Peak ($F_{1,20} = 5.50$, $P = 0.03$) (Fig. 1C). *Post hoc* comparisons revealed that post-intervention mean MEP amplitudes for the Trough protocol were significantly reduced at the 10-min time point compared with Sham (paired $t_{20} = 3.49$, $P = 0.002$), and at the 10 and 30-min time points compared with Peak (for all, paired $t_{20} \geq 2.13$, $P \leq 0.05$). When averaged across all post-intervention time points, **absolute** MEP amplitudes following the Trough tACS-cTBS protocol were reduced by ~22% compared with baseline (paired $t_{20} = 3.27$, $P = 0.004$). Conversely, there were no significant changes in

absolute MEP amplitudes following either the Peak or Sham tACS-cTBS protocols: grand-averaged **absolute** MEP amplitudes were increased by ~5% compared with baseline following the Peak tACS-cTBS protocol (paired t_{20} = -0.69, P = 0.50) and reduced by just 3% following the Sham protocol (paired t_{20} = 0.45, P = 0.66).

Alpha power is not associated with tACS-cTBS response

In the subset of 19 participants with available EEG, baseline alpha power did not differ between experimental sessions ($F_{2,36}$ = 0.24, P = 0.79), and was therefore averaged across sessions. Since the distribution of participants' average baseline alpha power differed significantly from normality (D_{19} = 0.20, P = 0.05), these data were log-transformed for regression analyses. Two participants were identified as outliers for the regression between alpha power and peak tACS-cTBS response (Cook's distance > 400% of the group mean for both), and were therefore excluded from this analysis. There was no influence of baseline alpha power on the tACS-cTBS response for either the Peak or Trough protocols (Fig 2A).

Higher *peak alpha frequency is associated with a greater Trough tACS-cTBS response*

Individual peak alpha frequency (IAF) at baseline did not differ between experimental sessions ($F_{2,36}$ = 2.23, P = 0.12), and was therefore averaged across sessions (mean: 9.6 Hz; range: 8–13.5 Hz). Following the exclusion of a single outlier (Cook's distance > 600% of the group mean for both Peak and Trough), while there was no association between average IAF and the tACS-cTBS response for the Peak protocol, a positive linear relationship was observed between average IAF and the Trough tACS-cTBS response (R^2 = 0.26, $F_{1,16}$ = 5.48,

$P = 0.03$), with **higher** peak alpha **frequency** associated with a greater difference between Trough and Sham conditions (Fig. 2B).

Trough tACS-cTBS induced MEP depression is greater in cTBS 'non-responders'

11 of the 21 participants responded with the expected decrease in MEP amplitudes following cTBS for the Sham tACS protocol. Comparison of stimulus intensities and baseline MEPs (averaged across all experimental sessions) showed no differences in RMT (independent $t_{19} = -1.16$, $P = 0.26$), SI_{1mV} (independent $t_{19} = -1.13$, $P = 0.27$), or baseline MEP amplitude (independent $t_{19} = 0.18$, $P = 0.86$) between cTBS responders and non-responders. Similarly, in the 19 participants with available EEG (9 responders, 10 non-responders), log-transformed average baseline alpha power did not differ between groups (independent $t_{17} = -0.04$, $P = 0.97$). However, cTBS non-responders had **higher** peak alpha **frequencies** than cTBS responders (independent $t_{17} = -2.85$, $P = 0.01$) (Fig. 3A).

A mixed-design ANOVA comparing normalised post-intervention MEP data between cTBS responders and non-responders showed a main effect of PROTOCOL ($F_{2,38} = 6.13$, $P = 0.02$), as well as a PROTOCOL*GROUP interaction ($F_{2,38} = 7.82$, $P = 0.007$). While post-intervention MEP amplitudes did not differ between protocols in responders ($F_{2,20} = 2.35$, $P = 0.15$), a significant effect was observed in non-responders ($F_{2,18} = 8.26$, $P = 0.01$), with greater MEP depression for the Trough protocol compared with both Sham ($F_{1,9} = 61.01$, $P < 0.001$) and Peak ($F_{1,9} = 6.33$, $P = 0.03$) (Fig. 3B). *Post hoc* comparisons on data from non-responders revealed that post-intervention mean MEP amplitudes for the Trough protocol were significantly reduced at the 0, 5, 10, and 30-min time points compared with Sham (for

all, paired $t_9 \geq 3.73$, $P \leq 0.005$), and at the 5, 10, and 30-min time points compared with Peak (paired $t_9 \geq 2.78$, $P \leq 0.02$).

Discussion

The present study demonstrates a novel approach of applying cTBS with bursts of magnetic stimuli time-locked to different phases of a tACS-imposed alpha oscillation. We show that the neuroplastic response to cTBS was enhanced when stimulus bursts were delivered in-phase with the trough, but not the peak, of the applied current. This enhancement of the cTBS response was dependent on the individual peak frequency of the endogenous alpha rhythm, and was strongest in participants that did not respond in the expected manner to cTBS alone.

Whereas the initial study by Huang *et al.* (2005) showed a lasting depression of MEP amplitudes following cTBS, we were unable to show a significant group-level response to cTBS in the Sham tACS condition. This is likely attributable to the considerable variability between subject response profiles, which is common for plasticity-inducing NIBS protocols and is caused by a number of factors (Ridding & Ziemann, 2010). Several recent studies have shown highly variable responses to cTBS, with little or no effect observed when averaged across individuals (Zafar *et al.*, 2008; Todd *et al.*, 2009; Di Lazzaro *et al.*, 2011; McAllister *et al.*, 2011; Goldsworthy *et al.*, 2012a; Goldsworthy *et al.*, 2012b; Hamada *et al.*, 2013; Vallence *et al.*, 2013; Brownjohn *et al.*, 2014). The results of the present study are consistent with these more recent reports.

One factor that might contribute to the large inter-subject response variability to cTBS and other plasticity-inducing NIBS protocols is the level of cortical inhibition at the time of stimulation. There is considerable evidence suggesting cortical inhibition is an important regulator of plasticity. Experiments in animal models have shown that LTP/LTD induction is enhanced during pharmacological blockade of gamma-amino butyric acid (GABA) receptor activity (Hess & Donoghue, 1994; Hess *et al.*, 1996). Similarly, in humans, both NIBS-induced plasticity and practice-dependent plasticity are enhanced when GABAergic inhibition is reduced (Ziemann *et al.*, 1998; Ziemann *et al.*, 2001), and diminished when inhibition is up-regulated by GABA receptor agonists (Butefisch *et al.*, 2000; Ziemann *et al.*, 2001; McDonnell *et al.*, 2007). Oscillatory activity in the alpha band is thought to reflect pulsatile inhibition of neural activity (Jensen & Mazaheri, 2010; Mathewson *et al.*, 2011), and given the influence of inhibition on plasticity induction, we hypothesised that applying cTBS bursts on different phases of the alpha rhythm would modulate the plasticity response. Previous studies have shown evidence that tACS can be used for the specific entrainment of endogenous cortical rhythms, inducing frequency-dependent effects on cortical processing and excitability when applied to visual (Kanai *et al.*, 2008; Kanai *et al.*, 2010; Zaehle *et al.*, 2010; Helfrich *et al.*, 2014; Strüber *et al.*, 2014) and motor systems (Antal *et al.*, 2008; Pogosyan *et al.*, 2009; Feurra *et al.*, 2011; Brittain *et al.*, 2013). Here, we show that 10 Hz tACS modulates the plasticity response to cTBS in a phase-specific manner, promoting MEP depression when bursts were time-locked to the troughs, but not peaks, of the exogenous alpha rhythm.

Based on the perceived role of cortical inhibition in the generation of alpha oscillatory activity, it seemed plausible that alpha band power would influence the plasticity response to combined tACS-cTBS in the present study. However, no relationship was observed between

baseline alpha power and the response to either Peak or Trough tACS-cTBS. It is possible this was due to the simple bipolar electrode montage used for recording EEG in this study, with neuronal activity from widespread cortical sources not limited to the targeted motor cortical region contributing to spectral power in the alpha frequency band. This result is consistent with a previous study of ours in which we were also unable to demonstrate a relationship between baseline EEG power (measured with a similar bipolar montage) and the plasticity response to cTBS (McAllister *et al.*, 2011).

In contrast to baseline alpha power, having a **higher** peak alpha **frequency** was associated with a greater plastic response to the Trough tACS-cTBS protocol. The peak frequency of endogenous alpha also appeared to be an important determinant of the response to cTBS in the Sham tACS condition, with those participants that responded with the expected decrease in MEP amplitudes (i.e., cTBS 'responders') having a significantly **lower** peak alpha **frequency** compared with those that showed the opposite response (i.e., cTBS 'non-responders'). Although the mechanisms underpinning this relationship are unclear, several previous studies have observed functional differences between the lower and upper alpha frequency bands. **Higher** alpha **frequencies are** associated with more task related and topographically distinct cognitive processing compared with **lower** alpha, which is topographically widespread and likely reflects non-task related attentional demands (Klimesch *et al.*, 1994; Klimesch *et al.*, 1997a; Klimesch *et al.*, 1997b; Doppelmayr *et al.*, 2002). A similar dissociation has been observed between the reactivity patterns of alpha originating from sensorimotor cortical areas (mu rhythms) in response to movement execution and motor imagery, with somatotopically focused desynchronisation of faster mu rhythms that is specific to the movement performed/imagined (Pfurtscheller *et al.*, 2000; Pfurtscheller *et al.*, 2006). Therefore, one possible explanation for our results is that the

higher peak alpha **frequencies** observed in cTBS non-responders may represent high levels of localised inhibition, whereas the **lower** alpha **frequencies** in cTBS responders may be reflective of more generalised low-level inhibition.

Interactions between the peak frequency of endogenous cortical rhythms and the plasticity response to cTBS have been investigated previously. Brownjohn *et al.* (2014) applied both cTBS and the facilitatory intermittent TBS with inter-burst frequency individualised to the dominant cortical rhythm, but were unable to demonstrate an improvement in response. Contrary to our findings, these authors observed no association between TBS response and the peak frequency of cortical rhythms recorded at baseline. While this may suggest that the variable response to TBS is unrelated to the frequency of cortical oscillatory activity at the time of stimulation, it should be noted that the influence of phase was not examined. Furthermore, the dominant cortical rhythms observed in their study were predominately in the theta frequency band, and therefore, the influence of IAF on the TBS response was not tested.

It is unclear why non-responders to cTBS alone respond with stronger MEP depression to the Trough tACS-cTBS protocol in the present study compared with cTBS responders. We suggest two possible explanations: (1) the **higher peak** alpha **frequencies** observed in cTBS non-responders may be more susceptible to entrainment by 10 Hz tACS, and (2) the non-response to cTBS alone was due (in part) to high levels of localised cortical inhibition at the time of stimulation, and thus timing bursts of magnetic stimuli to the trough of the imposed alpha oscillation (that is, during periods when inhibition is presumably reduced) is more

likely to promote plasticity induction in these participants. Based on the current data, we are unable to speculate further on the underlying mechanisms for this effect.

While the enhanced neuroplasticity response following Trough tACS-cTBS could have been the result of entrainment of endogenous alpha, an alternate explanation for our findings is the potential influence of changing membrane potential on plasticity induction. It is possible that cTBS-induced MEP depression was enhanced when bursts of stimuli were applied at the trough of the tACS-imposed alpha oscillation because post-synaptic cell membranes were hyperpolarised at the trough, potentially reducing the level of Ca^{2+} entry and thus favouring the induction of LTD. Conversely, applying bursts of stimuli at the peak of the oscillation (that is, during maximal depolarisation of membrane potentials) might increase the level of Ca^{2+} entry, favouring LTP induction. Studies in both animal models and humans provide some evidence consistent with this phase-dependent induction of LTP and LTD (Wespatat *et al.*, 2004; Cash *et al.*, 2013). While we favour the alpha entrainment and pulsatile cortical inhibition hypothesis as the most likely explanation for the current results, additional studies would be necessary to provide additional support for the contribution of these mechanisms.

It should be noted that there are several limitations to this study. First, although the classification of responders and non-responders to cTBS was based on criteria similar to that used in previous studies investigating other plasticity-inducing NIBS protocols (Müller-Dahlhaus *et al.*, 2008; Delvendahl *et al.*, 2010; Delvendahl *et al.*, 2012; Voytovych *et al.*, 2012; Hamada *et al.*, 2013), this approach is somewhat arbitrary. While a cluster analysis performed on a larger sample would have allowed a clearer and more accurate distinction

between groups of participants, we were still able to show a significant difference between groups with respect to both IAF at baseline as well as the plasticity response to tACS-cTBS.

Second, real cTBS was used for each experimental protocol in this study, and as a result we cannot exclude the possibility that tACS alone is having a modulatory effect on MEP amplitudes. We consider this unlikely, however, since tACS was only applied for very short durations in this study (i.e., 40 s for the Peak and Trough protocols, and 0.3 s for the Sham protocol), and it has previously been shown that 5 min of 10 Hz tACS produced no lasting after-effects on MEP amplitudes when applied to the primary motor cortex (Antal *et al.*, 2008).

Finally, while we propose that an entrainment of endogenous alpha is the likely mechanism by which tACS influences the cTBS response, we have no direct evidence from the current data to support this. Although the simultaneous recording of EEG during tACS is technically challenging due to large artifacts, using a combination of artifact template subtraction and principal component analysis, Helfrich *et al.* (2014) were recently able to demonstrate entrainment of parietal-occipital alpha activity during a 10 Hz tACS protocol, with increases in alpha power as well as phase- and frequency-specific synchronisation to the externally applied stimulus. However, a much longer duration of stimulation was used by Helfrich *et al.* (i.e., 20 min, compared with the 40-s protocol used here), and the minimum duration required for entrainment to occur was not reported. Therefore, additional studies are required to determine whether the 40-s duration of 10 Hz tACS is capable of entraining endogenous alpha, and also, if this entrainment is causally related to the modulation of cTBS-induced cortical plasticity. Additionally, because EEG was not recorded during the stimulation period,

it is unclear what effect cTBS, either by itself or through interaction with tACS, might have had on the power and/or phase of ongoing alpha. Indeed, it has previously been shown that rTMS protocols, including cTBS, can influence the power and phase of cortical oscillations (Brignani *et al.*, 2008; Fuggetta *et al.*, 2008; Veniero *et al.*, 2011; Noh *et al.*, 2012). Therefore, this will also need to be addressed in future work.

This study demonstrates for the first time that the plasticity response to a non-invasive brain stimulation paradigm can be modulated in a phase-dependent manner when applied in combination with tACS. Specifically, we showed that the LTD-like neuroplastic response to cTBS (Huang *et al.*, 2007) was enhanced when bursts of stimuli were delivered in-phase with the trough, but not the peak, of an externally applied 10 Hz alpha oscillation. This LTD-like plasticity enhancement was only observed in those participants classified as non-responders to cTBS who, interestingly, exhibited a **higher** peak frequency of endogenous alpha at baseline. While further work is required to better understand the underlying mechanisms for this effect, these findings open up intriguing possibilities for both enhancing cTBS-induced LTD-like plasticity in clinical settings, and pre-selecting those individuals likely to benefit from combined tACS-cTBS based on their baseline EEG.

Acknowledgements

None of the authors have potential conflicts of interest to be disclosed. This work was funded by a National Health and Medical Research Council (NHMRC) project grant. MRG is supported by an AARDF Postdoctoral Fellowship.

Abbreviations

cTBS, continuous theta burst stimulation

EEG, electroencephalography

EMG, electromyography

FDI, first dorsal interosseous

GABA, gamma-amino butyric acid

IAF, individual peak alpha frequency

LTD, long-term depression

LTP, long-term potentiation

MEP, motor evoked potential

NIBS, non-invasive brain stimulation

RMT, resting motor threshold

tACS, transcranial alternating current stimulation

TMS, transcranial magnetic stimulation

References

Antal, A., Boros, K., Poreisz, C., Chaieb, L., Terney, D. & Paulus, W. (2008) Comparatively weak after-effects of transcranial alternating current stimulation (tACS) on cortical excitability in humans. *Brain Stimul*, **1**, 97-105.

Brignani, D., Manganotti, P., Rossini, P.M. & Miniussi, C. (2008) Modulation of cortical oscillatory activity during transcranial magnetic stimulation. *Human brain mapping*, **29**, 603-612.

Brittain, J.S., Probert-Smith, P., Aziz, T.Z. & Brown, P. (2013) Tremor Suppression by Rhythmic Transcranial Current Stimulation. *Current Biology*, **23**, 436-440.

Brownjohn, P.W., Reynolds, J.N., Matheson, N., Fox, J. & Shemmell, J.B. (2014) The effects of individualized theta burst stimulation on the excitability of the human motor system. *Brain Stimul*, **7**, 260-268.

Butefisch, C., Davis, B.C., Wise, S.P., Sawaki, L., Kopylev, L., Classen, J. & Cohen, L.G. (2000) Mechanisms of use-dependent plasticity in the human motor cortex. *Proc.Natl.Acad.Sci.U.S.A.*, **97**, 3661-3665.

Cash, R.F., Murakami, T., Chen, R., Thickbroom, G.W. & Ziemann, U. (2014) Augmenting Plasticity Induction in Human Motor Cortex by Disinhibition Stimulation. *Cereb Cortex*.

Cash, R.F.H., Mastaglia, F.L. & Thickbroom, G.W. (2013) Evidence for high-fidelity timing-dependent synaptic plasticity of human motor cortex. *Journal of neurophysiology*, **109**, 106-112.

Clarkson, A.N., Huang, B.S., MacIsaac, S.E., Mody, I. & Carmichael, S.T. (2010) Reducing excessive GABA-mediated tonic inhibition promotes functional recovery after stroke. *Nature*, **468**, 305-U193.

Cook, R.D. (1977) Detection of Influential Observation in Linear Regression. *Technometrics*, **19**, 15-18.

Delvendahl, I., Jung, N.H., Kuhnke, N.G., Ziemann, U. & Mall, V. (2012) Plasticity of motor threshold and motor-evoked potential amplitude--a model of intrinsic and synaptic plasticity in human motor cortex? *Brain Stimul*, **5**, 586-593.

Delvendahl, I., Jung, N.H., Mainberger, F., Kuhnke, N.G., Cronjaeger, M. & Mall, V. (2010) Occlusion of bidirectional plasticity by preceding low-frequency stimulation in the human motor cortex. *Clin Neurophysiol*, **121**, 594-602.

Di Lazzaro, V., Dileone, M., Pilato, F., Capone, F., Musumeci, G., Ranieri, F., Ricci, V., Bria, P., Di Iorio, R., de Waure, C., Pasqualetti, P. & Profice, P. (2011) Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. *Journal of neurophysiology*, **105**, 2150-2156.

Doppelmayr, M., Klimesch, W., Stadler, W., Pöllhuber, D. & Heine, C. (2002) EEG alpha power and intelligence. *Intelligence*, **30**, 289-302.

Dugue, L., Marque, P. & VanRullen, R. (2011) The phase of ongoing oscillations mediates the causal relation between brain excitation and visual perception. *J Neurosci*, **31**, 11889-11893.

Feurra, M., Bianco, G., Santarnecchi, E., Del Testa, M., Rossi, A. & Rossi, S. (2011) Frequency-Dependent Tuning of the Human Motor System Induced by Transcranial Oscillatory Potentials. *Journal of Neuroscience*, **31**, 12165-12170.

Fuggetta, G., Pavone, E.F., Fiaschi, A. & Manganotti, P. (2008) Acute modulation of cortical oscillatory activities during short trains of high-frequency repetitive transcranial magnetic stimulation of the human motor cortex: a combined EEG and TMS study. *Human brain mapping*, **29**, 1-13.

Gentner, R., Wankerl, K., Reinsberger, C., Zeller, D. & Classen, J. (2008) Depression of human corticospinal excitability induced by magnetic theta-burst stimulation: evidence of rapid polarity-reversing metaplasticity. *Cereb Cortex*, **18**, 2046-2053.

Goldsworthy, M.R., Müller-Dahlhaus, F., Ridding, M.C. & Ziemann, U. (2014) Inter-subject Variability of LTD-like Plasticity in Human Motor Cortex: A Matter of Preceding Motor Activation. *Brain Stimul*.

Goldsworthy, M.R., Pitcher, J.B. & Ridding, M.C. (2012a) The application of spaced theta burst protocols induces long-lasting neuroplastic changes in the human motor cortex. *Eur J Neurosci*, **35**, 125-134.

Goldsworthy, M.R., Pitcher, J.B. & Ridding, M.C. (2012b) A comparison of two different continuous theta burst stimulation paradigms applied to the human primary motor cortex. *Clinical Neurophysiology*, **123**, 2256-2263.

Haegens, S., Nacher, V., Luna, R., Romo, R. & Jensen, O. (2011) alpha-Oscillations in the monkey sensorimotor network influence discrimination performance by rhythmical inhibition of neuronal spiking. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 19377-19382.

Hamada, M., Murase, N., Hasan, A., Balaratnam, M. & Rothwell, J.C. (2013) The role of interneuron networks in driving human motor cortical plasticity. *Cereb Cortex*, **23**, 1593-1605.

Helfrich, R.F., Schneider, T.R., Rach, S., Trautmann-Lengsfeld, S.A., Engel, A.K. & Herrmann, C.S. (2014) Entrainment of brain oscillations by transcranial alternating current stimulation. *Current biology : CB*, **24**, 333-339.

Hess, G., Aizenman, C.D. & Donoghue, J.P. (1996) Conditions for the induction of long-term potentiation in layer II/III horizontal connections of the rat motor cortex. *J Neurophysiol*, **75**, 1765-1778.

Hess, G. & Donoghue, J.P. (1994) Long-term potentiation of horizontal connections provides a mechanism to reorganize cortical motor maps. *J Neurophysiol*, **71**, 2543-2547.

Hoogendam, J.M., Ramakers, G.M. & Di Lazzaro, V. (2010) Physiology of repetitive transcranial magnetic stimulation of the human brain. *Brain Stimul*, **3**, 95-118.

Huang, Y.Z., Chen, R.S., Rothwell, J.C. & Wen, H.Y. (2007) The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin Neurophysiol*, **118**, 1028-1032.

Huang, Y.Z., Edwards, M.J., Rounis, E., Bhatia, K.P. & Rothwell, J.C. (2005) Theta burst stimulation of the human motor cortex. *Neuron*, **45**, 201-206.

Jensen, O. & Mazaheri, A. (2010) Shaping functional architecture by oscillatory alpha activity: gating by inhibition. *Frontiers in Human Neuroscience*, **4**.

Kanai, R., Chaieb, L., Antal, A., Walsh, V. & Paulus, W. (2008) Frequency-Dependent Electrical Stimulation of the Visual Cortex. *Current Biology*, **18**, 1839-1843.

Kanai, R., Paulus, W. & Walsh, V. (2010) Transcranial alternating current stimulation (tACS) modulates cortical excitability as assessed by TMS-induced phosphene thresholds. *Clinical Neurophysiology*, **121**, 1551-1554.

Klimesch, W., Doppelmayr, M., Pachinger, T. & Ripper, B. (1997a) Brain oscillations and human memory: EEG correlates in the upper alpha and theta band. *Neurosci Lett*, **238**, 9-12.

Klimesch, W., Doppelmayr, M., Schimke, H. & Ripper, B. (1997b) Theta synchronization and alpha desynchronization in a memory task. *Psychophysiology*, **34**, 169-176.

Klimesch, W., Schimke, H. & Schwaiger, J. (1994) Episodic and semantic memory: an analysis in the EEG theta and alpha band. *Electroencephalogr Clin Neurophysiol*, **91**, 428-441.

Komaki, A., Shahidi, S., Lashgari, R., Haghparast, A., Malakouti, S.M. & Noorbakhsh, S.M. (2007) Effects of GABAergic inhibition on neocortical long-term potentiation in the chronically prepared rat. *Neuroscience Letters*, **422**, 181-186.

Mathewson, K.E., Lleras, A., Beck, D.M., Fabiani, M., Ro, T. & Gratton, G. (2011) Pulsed out of awareness: EEG alpha oscillations represent a pulsed-inhibition of ongoing cortical processing. *Frontiers in Psychology*, **2**.

McAllister, S.M., Rothwell, J.C. & Ridding, M.C. (2011) Cortical oscillatory activity and the induction of plasticity in the human motor cortex. *Eur J Neurosci*, **33**, 1916-1924.

McDonnell, M.N., Orekhov, Y. & Ziemann, U. (2007) Suppression of LTP-like plasticity in human motor cortex by the GABAB receptor agonist baclofen. *Exp.Brain Res.*, **180**, 181-186.

Müller-Dahlhaus, J.F., Orekhov, Y., Liu, Y. & Ziemann, U. (2008) Interindividual variability and age-dependency of motor cortical plasticity induced by paired associative stimulation. *Exp Brain Res*, **187**, 467-475.

Noh, N.A., Fuggetta, G., Manganotti, P. & Fiaschi, A. (2012) Long lasting modulation of cortical oscillations after continuous theta burst transcranial magnetic stimulation. *PloS one*, **7**, e35080.

Pfurtscheller, G., Brunner, C., Schlogl, A. & Lopes da Silva, F.H. (2006) Mu rhythm (de)synchronization and EEG single-trial classification of different motor imagery tasks. *NeuroImage*, **31**, 153-159.

Pfurtscheller, G., Neuper, C. & Krausz, G. (2000) Functional dissociation of lower and upper frequency mu rhythms in relation to voluntary limb movement. *Clin Neurophysiol*, **111**, 1873-1879.

Pogosyan, A., Gaynor, L.D., Eusebio, A. & Brown, P. (2009) Boosting Cortical Activity at Beta-Band Frequencies Slows Movement in Humans. *Current Biology*, **19**, 1637-1641.

Ridding, M.C. & Ziemann, U. (2010) Determinants of the induction of cortical plasticity by non-invasive brain stimulation in healthy subjects. *Journal of Physiology-London*, **588**, 2291-2304.

Rossi, S., Hallett, M., Rossini, P.M. & Pascual-Leone, A. (2009) Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol*, **120**, 2008-2039.

Sale, M.V., Ridding, M.C. & Nordstrom, M.A. (2007) Factors influencing the magnitude and reproducibility of corticomotor excitability changes induced by paired associative stimulation. *Exp.Brain Res.*, **181**, 615-626.

Strüber, D., Rach, S., Trautmann-Lengsfeld, S., Engel, A. & Herrmann, C. (2014) Antiphasic 40 Hz Oscillatory Current Stimulation Affects Bistable Motion Perception. *Brain Topogr*, **27**, 158-171.

Todd, G., Flavel, S.C. & Ridding, M.C. (2009) Priming theta-burst repetitive transcranial magnetic stimulation with low- and high-frequency stimulation. *Exp.Brain Res.*, **195**, 307-315.

Vallence, A.M., Kurylowicz, L. & Ridding, M.C. (2013) A comparison of neuroplastic responses to non-invasive brain stimulation protocols and motor learning in healthy adults. *Neurosci Lett*, **549**, 151-156.

Veniero, D., Brignani, D., Thut, G. & Miniussi, C. (2011) Alpha-generation as basic response-signature to transcranial magnetic stimulation (TMS) targeting the human resting motor cortex: A TMS/EEG co-registration study. *Psychophysiology*, **48**, 1381-1389.

Voytovych, H., Krivanekova, L. & Ziemann, U. (2012) Lithium: a switch from LTD- to LTP-like plasticity in human cortex. *Neuropharmacology*, **63**, 274-279.

Wespatat, V., Tennigkeit, F. & Singer, W. (2004) Phase sensitivity of synaptic modifications in oscillating cells of rat visual cortex. *J Neurosci.*, **24**, 9067-9075.

Zaehle, T., Rach, S. & Herrmann, C.S. (2010) Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PloS one*, **5**, e13766.

Zafar, N., Paulus, W. & Sommer, M. (2008) Comparative assessment of best conventional with best theta burst repetitive transcranial magnetic stimulation protocols on human motor cortex excitability. *Clin.Neurophysiol.*, **119**, 1393-1399.

Ziemann, U., Corwell, B. & Cohen, L.G. (1998) Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *Journal of Neuroscience*, **18**, 1115-1123.

Ziemann, U., Muellbacher, W., Hallett, M. & Cohen, L.G. (2001) Modulation of practice-dependent plasticity in human motor cortex. *Brain*, **124**, 1171-1181.

Figure legends

Figure 1. (A) Experimental design. Dark grey rectangles designate blocks of 15 MEP trials, measured using single-pulse TMS. Intervention with tACS-cTBS included either Sham, Peak, or Trough protocols, applied in all participants in three separate, counterbalanced sessions. (B) Stimulation patterns used for the three tACS-cTBS protocols. tACS was applied with a 10 Hz sinusoidal wave with no DC offset at an intensity of 1 mA for either 300 ms (for Sham) or 40 s (for Peak and Trough). Arrows indicate delivery of single magnetic stimuli during the cTBS train. (C) Change in MEP amplitude (normalised to baseline) following the Sham (open circles), Peak (grey upward triangles), and Trough (black downward triangles) tACS-cTBS protocols. Data are the group mean (± SEM) from 21 participants. $^{\#}P < 0.05$ compared with Peak; $^{*}P < 0.05$ compared with both Peak and Sham.

Figure 2. Relationship between baseline EEG alpha **power and frequency,** and the tACS-cTBS response for the Peak and Trough protocols (n = 19). The Peak and Trough tACS-cTBS responses were measured for each participant by subtracting grand-averaged post-intervention MEP amplitudes (normalised to baseline) from those recorded for the Sham protocol. Thus, values > 0 indicate greater depression for Peak/Trough tACS-cTBS, relative to Sham. (A) Average baseline alpha power (log-transformed) was not associated with either the Peak or Trough tACS-cTBS response. (B) While there was no association between individual peak alpha frequency (IAF) and the Peak tACS-cTBS response, participants with **higher** IAF showed a greater Trough tACS-cTBS response. Regression analyses were performed on data excluding outliers (open circles).

Figure 3. (A) Baseline spectral power differences between those participants that showed the expected decrease in MEP amplitudes following cTBS in the Sham tACS condition ('responders'; black line), and those that showed either no change or an increase ('non-responders'; grey line). The inset shows the significant difference in individual peak alpha frequency (IAF) between cTBS responders (R) and non-responders (NR) ($^{\#}P < 0.05$). Power spectrums and IAF were averaged across the three experimental sessions for each participant. Data represent group means (± SEM) from the 19 participants with available EEG (n = 9 responders; n = 10 non-responders). (B) Change in MEP amplitude (normalised to baseline) following the Sham (open circles), Peak (grey upward triangles), and Trough (black downward triangles) tACS-cTBS protocols in responders (top) and non-responders (bottom). Data are group means (± SEM) from 21 participants (n = 11 responders; n = 10 non-responders). $^{\#}P < 0.05$ compared with Sham; $*P < 0.05$ compared with both Peak and Sham.