The effect of γ-tACS on working memory performance in healthy controls

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Abstract
Transcranial Direct Current Stimulation (tDCS) has been widely investigated for its potential to enhance cognition, and in particular working memory, however to date standard approaches to stimulation have shown only modest effects. Alternative, more specialised, forms of current delivery may be better suited to cognitive enhancement. One such method is transcranial Alternating Current Stimulation (tACS) which delivers stimulation at a specific frequency and has been shown to entrain endogenous cortical oscillations which underlie cognitive functioning. To date there has been no comparison of the effects of tACS to those of tDCS on cognitive enhancement. In a randomised repeated-measures study design we assessed the effect of gamma (γ)-tACS, tDCS and sham tDCS on working memory in 18 healthy participants who attended three sessions held at least 72 h apart. Pre- and post-stimulation working memory performance was assessed using the 2 and 3-back. Our findings indicated the presence of a selective improvement in performance on the 3-back task following γ-tACS compared with tDCS and sham stimulation. The current findings provide support for further and more detailed investigation of the role of γ-tACS as a more specialised approach to neuromodulation.

1. Introduction

Non-invasive brain stimulation techniques, such as transcranial Direct Current Stimulation (tDCS), are being increasingly utilised in the cognitive neurosciences (Jacobson, Koslowsky, & Lavidor, 2012). tDCS involves the application of a weak electrical current applied to the scalp using two surface electrodes (anode and cathode). This current alters the excitability of brain cells by shifting their membrane potentials in a de- or hyperpolarising direction, thus making them more or less likely to fire (Nitsche & Fregni, 2007). Stimulation of brain cells under the anode appears to increase brain activity, whereas stimulation under the cathode generally has the opposite effect (Jacobson et al., 2012). Of note, findings in the motor cortex indicate that polarity is dependent upon stimulation parameters such as duration of stimulation, for example while 20 min of anodal tDCS has been shown to increase cortical excitability (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013) the provision of 26 min of anodal stimulation has been shown to decrease excitability (Monte-Silva et al., 2013). The applicability of these findings outside the motor cortex is unclear, however there is evidence that 20 min of anodal stimulation to the prefrontal cortex is behaviourally enhancing (Hoy et al., 2013). In addition to its acute effects on membrane potential thresholds, tDCS has been shown to induce changes outlasting the period of stimulation which are believed to be due to the induction of neuroplastic processes such as Long Term Potentiation (LTP) and Long Term Depression (LTD) (Monte-Silva et al., 2013). These effects are consistent with the growing body of research showing that tDCS is able to induce post-stimulation enhancement in cognitive performance, with the majority of evidence to date in the domain of working memory (WM) (Brunoni & Vanderhasselt, 2014).

In addition to tDCS, there is also growing interest in alternative forms of current delivery such as transcranial Alternating Current Stimulation (tACS) (Antal & Paulus, 2013). While tDCS delivers an electrical current which travels in a constant unipolar direction, tACS delivers a current that alternates at a specified frequency back and forth between the electrodes (Antal & Paulus, 2013; Helfrich et al., 2014). Stimulation with tACS in the EEG range (conventionally: 0.1–80 Hz) is believed to directly modulate cortical oscillations, with a growing number of studies showing entrainment of
endogenous oscillations at the frequency of stimulation (Herrmann, Rach, Neuling, & Strüber, 2013). tACS has been shown to impact cortical excitability in the motor cortex in a seemingly frequency dependent manner (Feurra et al., 2013); with additional studies showing similar frequency dependent effects of tACS on motor behaviours, including speed of voluntary movement (Joudi, Jenkins, Brittain, Aziz, & Brown, 2012). Studies have also shown tACS is able to impact sensory, perceptual and cognitive processes (see Herrmann et al., 2013 for review). However, to date there has been considerably less tACS research in these areas compared with tDCS.

As stated above, there is a growing body of research indicating that non-invasive brain stimulation, and tDCS in particular, is able to enhance WM. WM is the ability to temporarily store, access, and manipulate information ‘in mind’ in order to facilitate higher level cognitive functions (for example language, learning, problem solving) (Baddeley, 2007). Improvements in WM have been shown to result in enhanced performance on tasks requiring more complex thought and action (Jaušovec & Jaušovec, 2012). Impairments in WM are also a core, and functionally disabling, symptom of numerous neurological and psychiatric conditions. As such, the ability of brain stimulation techniques to enhance WM has significant implications, from the development of treatments for cognitive dysfunction in patient populations such as schizophrenia, traumatic brain injury and dementia, to a potential means of improving broad cognitive function in healthy populations. However, recent meta-analyses have concluded that current protocols, namely anodal tDCS to the left dorsolateral prefrontal cortex (DLPFC), induce modest improvements at best in WM (Brunoni & Vanderhasselt, 2014; Hill, Fitzgerald, & Hoy, in press). In order to determine whether more robust effects are possible, investigation of alternative stimulation protocols, such as tACS, is required.

Limited research to date has explored optimising parameters for enhancing cognition with forms of non-invasive electrical stimulation, including investigations into the effects of dose (Hoy, Arnold, Emson, Daskalakis, & Fitzgerald, 2014; Hoy et al., 2013; Teo, Hoy, Daskalakis, & Fitzgerald, 2011), provision of tDCS concurrent with cognitive activity (Andrews, Hoy, Enticott, Daskalakis, & Fitzgerald, 2011) and, alternate forms of current delivery, the most promising of which appears to be transcranial Alternating Current Stimulation (tACS) (Jaušovec & Jaušovec, 2014; Jaušovec, Jaušovec, & Pahor, 2014; Santarnecchi et al., 2013). The ability of tACS to entrain endogenous oscillations at the frequency of stimulation, as described above, is significant as it theoretically allows, for the first time, more direct enhancement of processes underlying cognition and modulation of specific frequencies of oscillations potentially allowing ‘specialised’ enhancements of selective cognitive functions (Herrmann et al., 2013). WM, for example, is associated with synchronous activity across multiple frequency bands independently (i.e. theta, alpha, beta, and gamma) as well as with frequency-coupling (i.e. theta-nested-gamma) (Howard et al., 2003; Jensen & Colgin, 2007; Sauseng et al., 2009). However, there is also evidence for the particular relevance of specific frequency bands for aspects of WM, such as the positive association between gamma band activity (>40 Hz) and performance at higher WM loads in healthy populations (Basar-Eroglu et al., 2007; Honkanen, Rouhinen, Wang, Palva, & Palva, 2014; Howard et al., 2003; Roux, Wibral, Mohr, Singer, & Uhlhaas, 2012). Therefore, provision of tACS at gamma frequency (γ-tACS) may act to significantly improve WM capacity in a load dependent manner as opposed to what could be seen as the more general, and seemingly modest, effects of tDCS.

The aim of the current study was to investigate the effect of γ-tACS on performance of tasks of increasing WM load in healthy controls compared to those of an active comparator (standard tDCS) and a placebo control (sham tDCS). Firstly, it was hypothesised that γ-tACS and tDCS would result in significantly greater overall improvements in WM than sham, and that the degree of improvement seen with γ-tACS would be superior to tDCS. We further hypothesised that γ-tACS would show a greater improvement in the higher memory load, while the degree of improvement following tDCS would show no differentiation across load.

2. Method

2.1. Participants

18 healthy control participants were recruited into the study, 17 right handed. There were 9 males and 9 females, with an overall mean age of 29.3 (standard deviation = 7.65) and on average 16.23 years of education (standard deviation = 1.00). Exclusion criteria included a history of any psychiatric or neurological illness, any serious medical conditions, or current pregnancy. Suitability was determined via interview which included the administration of the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). Written consent was obtained from the participants prior to the commencement of the study. Ethical approval was granted by Monash University and the Alfred Hospital ethics committee.

2.2. Procedure

This was a randomised repeated-measures single-blind study design. Participants attended for three sessions which were held at least 72 h apart (see Fig. 1). Each session involved the provision of 20 min of γ-tACS, tDCS or sham tDCS. The anodal/active electrode was positioned over the F3 position and the cathode/reference over the right supraorbital region. This is the standard montage for left DLPFC stimulation (Nitsche et al., 2008). Concurrent with stimulation participants also undertook two 5 min blocks of the 2-back WM task (from 2.5 to 7.5 min and from 12.5 to 17.5 min). WM performance was assessed using the 2- and 3-back WM task both prior to and following stimulation. The three repeated sessions were randomised and counterbalanced across participants.

2.3. Electrical stimulation

Stimulation was applied with the Eldith Stimulator Plus manufactured by neuroConn GmbH, Ilmenau, Germany. The Eldith Stimulator Plus is able to deliver all three forms of stimulation (alternating current, direct current and sham) as required for the study. The coding and settings required for the three different conditions, however, did not allow for the experimenter to be blind to the condition, resulting in a single blind experimental design. With respect to the specific nature of the stimulation types, participants were only informed that they would receive two active and one sham stimulation session. Blinding questionnaires were conducted at the end of each session. Stimulation was delivered through two 35 cm² (7 × 5 cm) electrodes, each covered with a sponge pad soaked in saline (0.09%). Impedance limit on the Eldith was set at 55 kΩ, impedances during stimulation were between 5 and 20 kΩ.

2.3.1. γ-tACS

tACS was delivered at a current of between ~750 μA and +750 μA at a frequency of 40 Hz for a total of 48,000 cycles, or 20 min with no current offset. The lack of a current offset was chosen to avoid the impact of static polarity effects. The active electrode was placed over the left DLPFC, located using F3, with the reference electrode placed over the contralateral supraorbital area. The stimulator was set to fade in and out over the maximum
2.3.2. tDCS

Anodal tDCS was delivered at 2 mA for a period of 20 min, with a 120/15 s fade-in/fade-out. The anode was placed over the left DLPFC, located using F3 in accordance with the international 10–20 system of measurement (as is the standard for left DLPFC stimulation), while the cathode was placed over the contralateral supraorbital area (Nitsche et al., 2008).

Current density for \( \gamma \)-tACS was marginally reduced to allow for minimisation of phosphene induction, whilst still remaining above the established threshold required for membrane polarisation (Francis, Gluckman, & Schiff, 2003). The current density for a positive half wave of \( \gamma \)-tACS was 0.021 mA/cm with the equivalent current density for tDCS 0.028 mA/cm.

2.3.3. Sham tDCS

Sham stimulation began with a fade into a peak of 2 mA over 120 s, immediately followed by 30 s of constant current stimulation and a 15 s fade out. This is the standard blinding procedure for tDCS (Nitsche et al., 2008). The anodal electrode was again placed over F3 and cathode over the contralateral supraorbital area.

2.4. Working memory task: n-back

2.4.1. Pre- and post-stimulation task

A series of random letters A to J were presented consecutively (10 min; consisting of a 5 min block of 2-back and a 5 min block of 3-back). Participants were required to respond with a button press when the present letter was the same as the letter presented 2 trials earlier. Each block consisted of 130 trials containing 32 targets (24.6%). Each letter was presented for 500 ms with a 1500 ms delay between stimuli presentations. Order of the two n-back tasks (i.e. 2- and 3-back) were counterbalanced across sessions and participants.

2.4.2. Intra-stimulation task

A series of random letters A to J were presented consecutively, over two 5 min blocks of 2-back across the 20 min of stimulation. Participants were required to respond with a button press when the present letter was the same as the letter presented 2 trials earlier. Each intra-stimulation block consisted of 130 trials containing 25% targets. Each letter was presented for 500 ms with a 1500 ms delay between stimuli presentations.

Each participant undertook the 2-back four times per session and the 3-back twice per session. Over the three sessions this equated to a total of 12 blocks of 2-back and 6 blocks of 3-back. Alternate stimuli were used for each of these blocks.

2.5. Statistical analysis

The dependent variables were difference scores (post – pre)/pre for \( d \) prime and accurate reaction time. There was no significant differences at the pre time point across conditions for either \( d \) prime (2-back: \( F_{(2,34)} = 1.434, p = 0.252 \); 3-back: \( F_{(2,34)} = 1.403, p = 0.260 \)) or accurate reaction time (2-back: \( F_{(2,34)} = 2.661, p = 0.084 \); 3-back: \( F_{(2,34)} = 1.987, p = 0.153 \)). \( d \) prime is a discriminability index which takes into account the ability to correctly identify targets and to minimise false alarms, and has been shown to have high sensitivity (Haatveit et al., 2010). In order to address our initial hypothesis that \( \gamma \)-tACS and tDCS would result in significantly greater overall improvements in WM than sham, and that the degree of improvement seen with \( \gamma \)-tACS and tDCS would be superior to tDCS we conducted two 2 x 3 ANOVAs using linear contrasts, one for each dependent variable (i.e. \( d \) prime and accurate reaction time), with stimulation type (\( \gamma \)-tACS, tDCS, Sham) and WM load (2-back, 3-back) as within subject factors. We also conducted a priori analyses to further investigate the effect of stimulation type on WM load. These analyses are required in order to directly address the secondary hypothesis, that there will be differential effects of the two active stimulation conditions compared to sham as a function of WM load. Results were assessed using two-tailed tests with an alpha level of 0.05.
3. Results

Order effects analysis confirmed the effectiveness of the counterbalancing of sessions, with no significant session order effects seen in either $d$ prime ($F_{(2, 34)} = 1.387, p = 0.264$) or accurate reaction time ($F_{(2, 34)} = 1.039, p = 0.365$). We also undertook analysis of blinding effectiveness. Participants did not guess the stimulation condition better than chance across the three sessions: session one ($\chi^2(1, N = 17) = 0.529, p = 0.467$), session two ($\chi^2(1, N = 16) = 1.000, p = 0.317$) session three ($\chi^2(1, N = 13) = 0.692, p = 0.405$).

3.1. $d$ prime

Means and standard errors of $d$ prime difference scores are provided in Table 1. Means and standard errors for accuracy at each time point are provided in the appendices.

To address our initial hypothesis we conducted a $2 \times 3$ ANOVA to investigate the effect of stimulation condition and WM load on change in $d$ prime performance. While there were mean differences in performance across the stimulation conditions in the predicted direction, the main effect of stimulation did not reach significance ($F_{(1, 17)} = 2.272, p = 0.114$). There was however a trend level stimulation by load interaction ($F_{(2, 34)} = 3.323, p = 0.086$). In light of this, and our a priori hypotheses with respect to load, post hoc analyses were undertaken.

Post hoc analyses within stimulation conditions revealed greater improvement over time on the 3-back (0.4380) compared to the 2-back (−0.004) for γ-tACS; this was a statistically significant difference of 0.4383 (95% CI, 0.710–0.166), ($t_{(17)} = 3.401, p = 0.003$; Cohens $d = 1.05$). There were no differences across WM load for tDCS ($t_{(17)} = 1.182, p = 0.254$) or sham ($t_{(17)} = 1.423, p = 0.173$). See Fig. 2.

Post hoc analyses within WM load showed no significant effect of stimulation on $d$ prime for the 2-back ($F_{(1, 17)} = 0.325, p = 0.576$). There was, however a trend towards significance for the 3-back ($F_{(1, 17)} = 3.323, p = 0.085$); with pairwise comparisons revealing that γ-tACS resulted in a larger improvement in performance when compared to sham (mean difference = 0.294, $p = 0.085$) but not tDCS (mean difference = 0.233, $p = 0.225$). There was no difference between tDCS and sham (mean difference = 0.060, $p = 0.605$).

3.2. Reaction time

Means and standard errors for reaction time are provided in Table 1.

To address our initial hypothesis we again conducted a $2 \times 3$ ANOVA to investigate the effect of stimulation condition and WM load on accurate reaction time difference scores. There was neither an effect of stimulation ($F_{(1, 17)} = 0.247, p = 0.626$) nor a stimulation by load interaction ($F_{(1, 17)} = 0.299, p = 0.592$) on reaction time.

Table 1

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>2-back Mean ± se</th>
<th>3-back Mean ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td>tACS</td>
<td>−0.000 ±0.053</td>
<td>0.438 ±0.141</td>
</tr>
<tr>
<td>tDCS</td>
<td>0.060 ±0.060</td>
<td>0.205 ±0.099</td>
</tr>
<tr>
<td>Sham</td>
<td>0.028 ±0.051</td>
<td>0.144 ±0.077</td>
</tr>
</tbody>
</table>

Table 2

Means and standard errors of change [(post − pre)/pre] in $d$ prime and accurate reaction time for the 2- and 3-back for each stimulation condition.

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>2-back Mean ± se</th>
<th>3-back Mean ± se</th>
</tr>
</thead>
<tbody>
<tr>
<td>tACS</td>
<td>−0.001 ±0.026</td>
<td>0.050 ±0.051</td>
</tr>
<tr>
<td>tDCS</td>
<td>0.020 ±0.033</td>
<td>0.037 ±0.031</td>
</tr>
<tr>
<td>Sham</td>
<td>0.026 ±0.034</td>
<td>0.021 ±0.029</td>
</tr>
</tbody>
</table>

Fig. 2. Means and standard errors for change in $d$ prime [(post − pre)/pre] for 2-back and 3-back separately for each stimulation condition. (a) γ-tACS, (b) tDCS and (c) sham.

As per our a priori hypotheses potential load effects were explored further. Analyses within stimulation conditions also revealed no differences in reaction time for 3-back compared to 2-back following γ-tACS ($t_{(17)} = −0.542, p = 0.595$), tDCS ($t_{(17)} = −0.366, p = 0.719$) or sham ($t_{(17)} = 0.115, p = 0.910$). Additionally, there was no significant effect of stimulation on reaction time for either the 2-back ($F_{(1, 17)} = 0.024, p = 0.880$) or the 3-back ($F_{(1, 17)} = 0.355, p = 0.559$).

4. Discussion

We did not find a significant effect of stimulation type on degree of improvement for overall WM performance. However, as predicted, γ-tACS was found to significantly enhance WM as a function of load; with a significant and large improvement in 3-back $d$ prime following stimulation (Cohen’s $d = 1.05$). Also as
There were no differential effects of tDCS on WM load. There were no significant effects of any stimulation condition on accurate reaction time, indicating the lack of a speed-accuracy trade-off in the improved performance on the 3-back following γ-tACS. Overall, the findings from the current study provide preliminary evidence for γ-tACS preferentially improving WM performance at higher loads.

Our results are consistent with the body of existing research to date on the cognitive effects of TACS. For example, significant positive effects on cognition have been seen with TACS provided in the alpha (Helfrich et al., 2014), theta (Jaušovec & Jaušovec, 2014; Jaušovec et al., 2014; Polania, Nitsche, Korman, Batsikadze, & Paulus, 2012; Vosskuhl, Huster, & Herrmann, 2015) and gamma range (Santarnecchi et al., 2013). Of particular relevance to the current findings, Santarnecchi et al. (2013) found γ-tACS to selectively enhance participants’ performance on more complex cognitive tasks where greater cognitive load was required. Additionally, the majority of these TACS studies also reported improvements on measures of accuracy rather than reaction time. To the best of our knowledge, ours is the first study of the effects of TACS on cognition to include both an active comparator (tDCS) and a placebo control (sham stimulation). The lack of significant improvement with TDCS was unexpected and is potentially explained by our inclusion of an intra-stimulation task. The majority of studies to date that have found improved WM in healthy controls post-stimulation have not used an intra-stimulation task, while those that did have shown mixed results with two out of three studies failing to show post-stimulation effects on WM performance (Mulgren, Hoy, Daskalakis, & Fitzgerald, 2011; Ohn et al., 2008; Teo et al., 2011). A possible explanation for these findings is that the use of a WM task concurrent with TACS may interfere with the subsequent effects of stimulation. While state dependent effects of TDCS are readily acknowledged in the literature, the often reported contention that combination of TDCS with a behavioural task can enhance the effects has only sparse research support (Nitsche et al., 2008; Andrews et al., 2011). It is possible that there is in fact no additive gain of combining TDCS and a cognitive task in healthy controls, whereby TDCS will not provide any improvement above and beyond the practice effects from repeated task performance. This is consistent with the theory suggesting the effects of TDCS in this group are limited and do not improve performance beyond an individual’s innate capability (Hoy & Fitzgerald, in press; Hoy et al., 2013). The current findings, in addition to the mixed findings mentioned above, indicate the need for more systematic investigation of the state dependent effects of TDCS. We did however find a significant post-stimulation effect of γ-TACS, which was also provided with an intra-stimulation task. The difference in findings could be due to the posited divergent mechanisms of action between the two approaches.

The current findings, of a large and selective improvement in 3-back performance following γ-TACS (Cohens d = 1.05), is consistent with this form of stimulation selectively, and directly, modulating cortical oscillations which are known to substantially contribute to specific cognitive functions (Helfrich et al., 2014; Jaušovec & Jaušovec, 2014; Jaušovec et al., 2014; Santarnecchi et al., 2013). This direct modulation of cortical oscillatory activity, as opposed to TDCS which is posited to have a more indirect effect by influencing GABA-ergic activity and providing a more ‘optimal cortical environment’ for the modulation of oscillations, may make this stimulation approach less susceptible to the possibly interfering state dependent effects of an intra-stimulation task (Hoy et al., 2013; Stagg, Bachtilar, & Johansen-Berg, 2011; Stagg et al., 2009). As hypothesised, the proposed mechanism of action of TACS is also likely to be responsible for the specificity of effects seen in the current study. Representation of information in WM has been proposed to occur via the synchronous firing of groups of neurons at specific frequencies (Jensen, Kaiser, & Lachaux, 2007). The complexity of cognitive functions such as WM necessitates cortical oscillatory involvement from multiple frequency bands (i.e. theta, alpha, beta, and gamma), with independent contributions as well as frequency-coupling (i.e. theta-nested-gamma) (Howard et al., 2003; Sauseng et al., 2009). There is however evidence for greater importance of specific frequency bands for particular aspects of WM, with gamma band activity in particular associated with degree of cognitive load (Honkanen et al., 2014; Roux et al., 2012). In a recent study by Roux et al. (2012) gamma band activity was shown to relate to the number of items maintained in WM, with positive correlations between gamma and WM capacity and negative correlations between gamma and reaction time. These significant associations clearly indicated the facilitatory nature of increased gamma in the performance of WM tasks with higher cognitive load. This study also showed site specificity, whereby the relationship between gamma activity and number of items coded in WM was specific to the left DLPFC (i.e. BA9) (Roux et al., 2012). Therefore the results of the current study, γ-TACS to the left DLPFC resulting in load dependent WM enhancement, are consistent with the modulation of gamma activity via γ-TACS. The seeming ability of TACS to more directly modulate the brain activity of interest is therefore a potential explanation for the selectivity of the effects seen.

While our findings provide support for the ability of γ-TACS to induce improvements in WM preferentially at a higher load, there are a number of limitations to be considered. Even in light of the repeated measures design of the study and the large effect size seen, the sample size could be considered relatively modest. Future research should extend this work into larger samples and utilise neurophysiological assessments to allow direct assessment of the mechanisms of actions of TACS. The effect of TACS across a range of frequencies on WM performance should be investigated to determine whether the current findings represent a frequency dependent effect. In addition, there is the potential for the ‘reference’ electrode, particularly in the tACS condition, to effect brain activity and hence behavioural outcomes. Future research utilising neurophysiological outcomes, and field modelling, would also be highly informative for the assessment of these potential effects. Finally, in light of the therapeutic potential of γ-TACS, this form of stimulation should be investigated in patient populations particularly where cognitive impairments have been associated with dysfunctional cortical oscillatory activity, (i.e. in schizophrenia where gamma oscillations are heavily implicated in the cognitive symptoms of the illness) (Sun et al., 2011). Despite the limitations and future research required, this study provides preliminary evidence in support of tACS as a specialised cognitive enhancer.
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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bandc.2015.11.002.

References


