

Combining tDCS and Working Memory Training to Down Regulate State Rumination: A Single-Session Double Blind Sham-Controlled Trial

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Abstract Rumination has been associated with reduced working memory and dorsolateral prefrontal cortex activity. This study explored whether single session anodal transcranial direct current stimulation (tDCS) applied to the dorsolateral prefrontal cortex and/or working memory training can transiently ameliorate working memory and down regulate state rumination. Sixty-six participants were randomly allocated to three conditions: (1) control training + tDCS, (2) working memory training + sham tDCS and (3) working memory training + tDCS. Before and after manipulation participants performed working memory tasks and state rumination was measured with self-report and heart rate variability. Participants who received real tDCS were significantly faster in switching between information in working memory than participants who received sham tDCS. No effects on self-reported state rumination were found. However, both groups receiving working memory training showed a higher increase in heart rate variability than the control training group, indicating more adaptive self-regulation.

Keywords Transcranial direct current stimulation (tDCS) · Working memory · Cognitive training · Rumination · Heart rate variability (HRV)

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Introduction

Rumination is defined as persistent attention on the causes, symptoms and implications of one's negative mood (Nolen-Hoeksema 1991). It is a major risk factor for depression (Nolen-Hoeksema et al. 2008). Studies looking at cognitive mechanisms associated with rumination have observed an important role of impaired operations in working memory (Berman et al. 2011; Joormann and Gotlib 2008; Koster et al. 2013). Working memory consists of systems of short-term storage for both auditory and visual information and a central executive that exerts attentional control on the content of working memory (Baddeley and Hitch 1974). At the neural level, several studies have identified the dorsolateral prefrontal cortex (DLPFC) as a key structure involved in the functioning of working memory (Cabeza and Nyberg 2000; Curtis and D'Esposito 2003). Several authors suggested that these cognitive impairments are not merely a correlate, but can act as etiological and maintaining factors in rumination (De Raedt and Koster 2010; Joormann 2005). For instance, impaired updating of the contents of working memory decreases the ability to appropriately remove irrelevant negative cognitions and memories from working memory, which in turn makes it difficult to attend to and process new information. Hence impaired working memory prolongs the activation of negative thoughts and facilitates sustained rumination (Joormann and Gotlib 2008).

In order to test whether working memory causally influences rumination, experimental procedures manipulating working memory and investigating the subsequent effects on rumination are required. Jaeggi et al. (2008) attempted to ameliorate working memory by means of the dual *n*-back training and observed that working memory capacity could be improved after 8 days of training. In this

training participants monitor visual and audio stimuli simultaneously and compare whether these match the stimuli n positions back. These results suggest that the n -back training could be a valuable tool to manipulate working memory.

Another interesting technique to influence working memory is transcranial Direct Current Stimulation (tDCS). TDCS is a method of neuromodulation in which a weak direct current is applied. This technique changes the excitability of the neurons: anodal stimulation causes a depolarization of the resting membrane potential, making the neurons more excitable, whereas cathodal stimulation causes a hyperpolarization of the resting membrane potential, making the neurons less excitable (Nitsche et al. 2008). Anodal tDCS on the DLPFC has proven successful in enhancing working memory capacity (for a meta-analysis Brunoni and Vanderhasselt 2014). Furthermore, Richmond et al. (2014) showed that combining working memory training and anodal tDCS on the DLPFC can enhance learning on the verbal part of the training task and facilitate near transfer to other untrained working memory tasks in comparison to a test–retest control group.

Based on the interesting findings of combining tDCS with working memory training, we examined whether anodal tDCS on the DLPFC in combination with a single-session working memory training provides a suitable method to induce a transient improvement of working memory in order to explore the causal influence of working memory on rumination. More precisely we hypothesized that the effect of working memory training can be facilitated by heightened activation of the DLPFC. Therefore combining tDCS with working memory training should lead to better working memory functioning and decreased state rumination. For this purpose, we combined a single session working memory training procedure (Jaeggi et al. 2008) with neuromodulation of the DLPFC using tDCS. Three conditions were used: (1) control training + tDCS, (2) working memory training + sham tDCS and (3) working memory training + tDCS.

We examined the effects of these manipulations on a non-emotional and an emotional working memory task. First, we expected effects on the non-emotional working memory task through the combination of tDCS and working memory training. The hypothesis whether the effect of the manipulation is similar or stronger for emotional working memory than general working memory is of an exploratory nature. For instance Corbetta and Shulman (2002) pose that attentional control is controlled by a bottom-up system and a top-down system. The top-down system is based on knowledge, current goals, and expectations and helps us to focus on the task at hand. The stimulus-driven system enables us to flexibly adapt to the environment by directing our attention to salient stimuli.

Incorporating emotional stimuli such as angry faces in a cognitive task can activate the bottom-up system (Eysenck et al. 2007). Since working memory is a limited capacity system, this means there are less resources for top-down control, which could subsequently impair performance on the cognitive task itself. Manipulating general working memory through tDCS and working memory training could strengthen top-down control. This could reduce the influence of the bottom-up system and thus diminish the distracting effects of emotional stimuli. However when emotional stimuli are relevant to the task, the bottom-up system and top-down system could interact resulting in enhanced working memory for emotional material (Eysenck et al. 2007). The inclusion of an emotional working memory task enabled the current study to determine if working memory training, either alone or combined with tDCS, differentially influences general and emotional working memory.

Finally, we hypothesized that transient improvement of working memory will enable participants to inhibit rumination during a 10 min rest period, shown by reduced self-reported rumination and higher heart rate variability (HRV). For this purpose we used a design measuring working memory and state rumination pre- and post-manipulation (i.e., tDCS + control training, sham tDCS + working memory training or tDCS + working memory training). Two 10 min rest periods (pre- and post-manipulation) were used to examine state rumination. Rest is a naturalistic setting which elicits heightened levels of rumination (Marchetti et al. 2013). State rumination was assessed using self-report measures. Since self-report measures are susceptible to demand effects and rumination has been associated with HRV (e.g., Ottaviani et al. 2009; Woody et al. 2014), HRV was included as a dependent variable to measure the physiological correlate of rumination. HRV refers to the variation in inter-beat-intervals between normal heartbeats. Ottaviani et al. (2009) suggested that rumination has the effect of temporarily taking the prefrontal cortex “off-line” with the consequence of a disinhibition of the sympathoexcitatory neural circuits and thus parasympathetic withdrawal (i.e., low HRV).

Methods

Participants

The sample included 66 healthy participants (13 males, 53 females) ranging in age from 18 to 48 years ($M = 23.09$, $SD = 5.03$). Participants from Ghent University and the surrounding communities were recruited online. Exclusion criteria were: (a) current or past heart, respiratory, neurological problems, (b) current depressive disorder, (c) current

use of psychotropic medications, (d) currently engaged in any form of psychological or psychiatric treatment, (e) poor vision and (f) pregnancy at the time of testing. These criteria were checked through an interview and the MINI-screen (Sheehan et al. 1998) at the beginning of the experiment. The study was approved by the medical ethical committee at Ghent University. Informed consent was obtained from all individual participants included in the study. Participants were paid 25 euro for their contribution.

Transcranial Direct Current Stimulation

Transcranial Direct Current Stimulation (tDCS) was employed in order to modulate the DLPFC and enhance working memory. Direct current stimulation was applied by a pair of surface sponge rubber electrodes (25 cm²) soaked in saline and was administered by a DC-stimulator (Neuroconn, Ilmenau, Germany). The decision on the size of the electrodes was informed by the study of Bai et al. (2014) in which they found that the use of a smaller electrode at F3 increased the E-field at the left DLPFC. The DLPFC was stimulated by placing the anode electrode centered over F3 according to the 10–20 international system for electroencephalogram electrode placement. This electrode placement and method of DLPFC localization is in accordance with previous tDCS studies over the left DLPFC looking at working memory processes (Fregni et al. 2005; Martin et al. 2013; Zaehle et al. 2011). In the current study the cathode was placed over the contra lateral supra orbital area. A constant current of 2 mA intensity was applied for 25 min with 30 s ramping up and down of current. Sham tDCS montage was exactly the same as during tDCS stimulation, however the current was ramped down after 30 s. This procedure is often used by tDCS researchers and has been found to be a near-optimal and reliable sham condition (Brunoni et al. 2011). In the current study all participants considered tDCS tolerable and did not express the need to stop tDCS. However some participants did report mild side effects such as slight itching and tingling under the electrodes and a few participants reported a headache. These side effects disappeared immediately after discontinuation of tDCS.

Heart Rate Variability Measurement

Heart rate variability (HRV) was used to measure the physiological correlate of rumination in order to test whether rumination decreases after tDCS and working memory training. Heart rate was measured beat-to-beat during a rest period before and after experimental manipulation with a telemetric heart rate monitor (Polar RS800CX). The heart rate data were transmitted to a personal computer and artifacts were filtered with Artiifact (Kaufmann et al. 2011). Time domain analyses and frequency domain analyses are

the main methods to calculate HRV. In the time domain analysis the square root of the mean of the sum of the squares of differences between adjacent RR intervals (RMSSD) was used to measure HRV (Malik 1996). In the frequency domain RR intervals are calculated by the frequency of the fluctuation. In this study, the high frequency (HF) power method was used. HF fluctuations are caused by respiration and are effected by vagal activity or parasympathetic modulation. This index has been shown to decrease as a function of stress (Berntson and Cacioppo 2004). The low frequency power and the LF/HF ratio were not calculated because of the difficulty to interpret these measures (Malik 1996). The employed methods are recommended for short-term components of HRV (Malik 1996). In accordance with recommendations (Malik 1996), 5 min of each relax period were selected for the best quality data. If the entire 10 min were of sufficient quality it was decided to exclude the first 3 min and the last 2 min. In 19 % of the data there were too many artifacts within this timeframe. In these cases extra HRV data of the last 2 min were used as a substitute for the artifacts.

Materials

MINI-Screen

The Dutch version of the MINI International Neuropsychiatric Interview-screen (MINI-screen; Sheehan et al. 1998) was used to check participants for psychiatric symptoms such as depression. This structured interview consists of several questions assessing both current and lifetime psychiatric diagnoses based on the DSM-IV. This interview is a valid and reliable measure of psychiatric diagnoses (Sheehan et al. 1998).

Rumination Response Scale

A Dutch version of the Rumination Response Scale (RRS; Nolen-Hoeksema and Morrow 1991; Raes et al. 2009; Treynor et al. 2003) was administered to assess trait tendencies to ruminate. It consists of 22 items assessing ruminative thoughts and actions in response to a depressive mood that are focused on the self, symptoms or consequences of that mood. Participants respond on a 4-point Likert scale ranging from 1 (almost never) to 4 (most of the time). Raes et al. (2009) showed that the RRS is a reliable and valid measure of rumination in Dutch speaking populations.

Momentary Ruminative Self-Focus Inventory

The Momentary Ruminative Self-focus Inventory (MRSI; Mor et al. 2013) was administered to measure momentary rumination before and after a 10-min rest period before and after experimental manipulation (i.e., tDCS + control

training, sham tDCS + working memory training or tDCS + working memory training) in order to test whether rumination decreases after tDCS and working memory training. The MRSI is a valid and reliable measure of momentary ruminative self-focused responses (Mor et al. 2013). It consists of 6 statements, for instance “right now, I wonder why I react the way I do” and “right now, I am conscious of my inner feelings”. Participants indicate to what extent they endorse these statements on a 7-point Likert scale ranging from 1 (strongly disagree) to 7 (strongly agree).

Profile of Mood States

A shortened Dutch version of the Profile of Mood States (POMS; McNair et al. 1971; Wald and Mellenbergh 1990) was administered at the beginning and end of the experiment to assess changes in mood. The POMS consists of 32 adjectives to which participants respond on a Likert scale ranging from 0 (not at all) to 4 (extremely). The 32 adjectives are distributed over five categories: depression, tension, anger, vigor and fatigue. The reliability and validity of this version has proven sufficient (Wicherts and Vorst 2004).

Running Span Task

In order to test whether general working memory accuracy increased after tDCS and working memory training, general working memory accuracy was assessed with the Running Span Task (RSpan; Broadway and Engle 2010) before and after manipulation. The RSpan task was programmed by Nitz (2010) in accordance with Broadway and Engle (2010). It ran using the inquisit software package (Draine 2011) on a Windows 8 computer with a 17 inch monitor. Participants were instructed to report the last n letters in the correct order of a series of sequentially presented letters (a combination of F, H, J, K, L, N, P, Q, R, S, T, or Y). The letters were presented in the center of the screen in black 18-pt font against a gray background for 300 ms with an interstimulus interval of 200 ms. The length of the n letters to be reported varied from three to six and were blocked in a random order. The number of irrelevant letters (varying from zero to two) preceding the targets was randomized within blocks. More precisely, one trial for each target length presented a series in which zero, one or two irrelevant letters preceded the targets. At the start of the block, participants were informed on how many letters they had to report from each list in that block. After each series the participants reported the letters by clicking the cells of a 3×4 grid displaying all possible letters. The response screen reminded participants how many letters they had to report. Participants received one point for every

letter that was correctly reported in the correct serial position.

Internal Shift Task

In order to test whether speed of working memory and emotional aspects of working memory increased after tDCS and working memory training the Internal Shift Task (IST; Chambers et al. 2008; De Raedt and Koster 2010) was employed. The IST was programmed using the E-prime 2.0 software package (Psychology Software Tools Inc, 2007) by De Lissnyder et al. (2012a) and ran on a Windows 7 laptop with a 15.6 inch monitor. In this task a series of a random number between 10 and 14 angry and neutral faces of males and females were presented sequentially per block. These pictures were based on a validation (Goeleven et al. 2008) of the Karolinska Directed Emotional Faces (Lundqvist et al. 1998). This task consisted of an emotion condition and a gender condition. In the emotion condition participants were instructed to keep a mental count of the amount of neutral and angry faces. In the gender condition instructions were identical for male and female faces. The order of the conditions was randomized over participants. During the presentation of every face participants updated one of the counters (for example from two to three angry faces in the emotion condition), but in their working memory they always had to sum the counter they adapted as well as the counter that stayed the same (for instance from two neutral faces and two angry faces to two neutral faces and three angry faces). When participants updated one of the counters (neutral-angry in the emotion condition and male-female in the gender condition), they were required to press the spacebar as fast as possible after every face presented. The latency to press the spacebar was employed as a measure of the latency to update the counters in the working memory. At the end of a block participants were asked to report the number of faces of both categories (neutral-angry in the emotion condition and male-female in the gender condition). In the emotion condition participants always had to report the number of neutral faces first and the angry faces after and in the gender condition male faces first and female faces after. This was done to encourage a consistent counting strategy. Each condition had a practice phase (3 blocks of trials) and an experimental phase (12 blocks of trials). In the experimental phase participants counted in silence.

The most important outcome measure of this task is the reaction time on switch and no-switch trials. A trial is considered a switch trial when participants have to update a different category than the preceding trial (i.e., angry when the previous face was neutral in the emotion condition). No-switch trials are trials in which participants have to

update the same category as the preceding trial (i.e., female when the previous face was also female in the gender condition). Switch trials require more cognitive effort and therefore are more sensitive to changes in working memory processes. Moreover, switching between specific types of information in working memory may be a key process underlying the ability to disengage from ruminative thinking.

Dual n-Back Training

In order to train working memory the dual *n*-back training was used. The dual *n*-back training ran using the Brainworkshop software package (Hoskinson and Toomim 2008) on a Windows 8 computer with a 17 in. monitor. In this task (Jaeggi et al. 2008) a blue square was presented sequentially on different locations in a 3×3 grid with a stimulus exposure duration of 500 ms and an interstimulus interval of 2500 ms. Participants were simultaneously presented auditorily with a letter (C, H, K, L, Q, R, S or T) through computer speakers. The location of the square and the letter were determined randomly. Participants were required to press the key Q with their left index finger on an AZERTY keyboard when a blue square appeared on the same location as *n* positions back in the sequence and the key L with their right index finger when the spoken letter was the same as the one presented *n* positions back. A block consisted of $20 + n$ combinations of a letter and square. The value of *n* started at $n = 2$, meaning for the first four presentations participants had to compare the third presented square and letter to the ones presented first, the fourth presented square and letter to the ones presented second etc. When performance accuracy at the end of a block reached 90 % or higher, *n* was raised by one. On the contrary, when performance accuracy was lower than 75 %, *n* was decreased by one. The lowest possible *n* was one, in which participants had to compare the current square and letter to the previous square and letter. In other cases *n* remained unchanged. In the current study participants completed 20 blocks of training.

Position and Sound 1-Back

For the purpose of a control condition, participants in the control training group were randomly allocated to either the position 1-back or the sound 1-back. This task resembled the dual *n*-back training, but required minimal efforts of working memory. The position and sound 1-back ran using the Brainworkshop software package (Hoskinson and Toomim 2008) on a Windows 8 computer with a 17 in. monitor. Participants were only required to monitor one stream of stimuli. In the position 1-back, participants reported whether the current square was the same as the

square presented before by pressing the key Q on an AZERTY keyboard. No letters were presented auditorily during this task. In the sound 1-back task participants were presented with the same 3×3 grid with a blue square always presented in the middle. Simultaneously, letters were presented auditorily through computer speakers. In this version of the task participants responded by pressing the key L on an AZERTY keyboard when they heard a letter that was the same as the one presented before. The *n* did not increase or decrease as a function of performance accuracy per block. Parallel to the *n*-back training, participants completed 20 blocks.

Procedure

We refer to Fig. 1 for an overview of the total procedure. Participants were randomly assigned to one of three groups in a double blind design: (1) real tDCS in combination with a control training (CT + tDCS); (2) placebo tDCS in combination with a working memory training (WMT + sham tDCS); (3) working memory training in combination with real tDCS (WMT + tDCS). After completing the informed consent form, participants were connected to the polar heart rate monitor belt and watch. HRV data was collected during the whole experiment. Subsequently the MINI-screen and the questionnaires were administered. Next participants performed on the first RSpan. Then participants filled out the first MRSI and were asked to relax for a period of 10 min. After the rest period participants filled out the second MRSI. Subsequently electrodes were soaked in saline solution and placed on the participant's scalp using the electrode montage described above. Following 5 min of stimulation (tDCS or sham) participants started with the working memory or control training for the remainder 20 min of stimulation. A 5 min period was used before the training to allow time for the tDCS to have its effect. After stimulation, the tDCS device was removed and participants performed the second RSpan followed by the IST. Next participants were administered the third MRSI and instructed to rest for 10 min. After the rest period participants filled out the last MRSI and the second POMS. Participants were fully debriefed at the end of the study.

Statistical Analysis

Statistics were performed using SPSS (version 20; IBM Corp, 2011) at a significance level of .05. Effect sizes are reported in the form of partial eta-squared (η_p^2). Following the recommendation of Elliott and Hawthorne (2005), missing values in repeated measures were imputed by substituting it with data of the closest match. If matches were equally close, the mean of the closest matches was substituted. One participant in the working memory

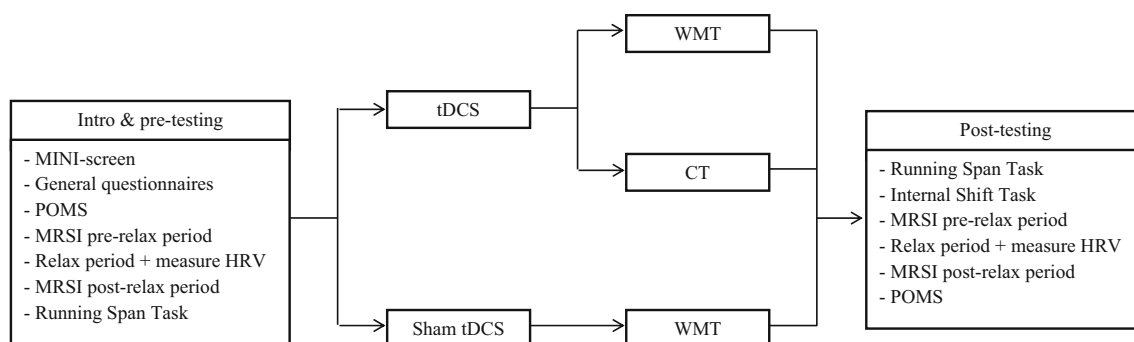


Fig. 1 Overview of the procedure (POMS Profile of Mood Scales, MRSI Momentary Ruminative Self-focus Inventory, HRV heart rate variability, tDCS transcranial direct current stimulation, WMT working memory training, CT control training)

training + tDCS group met criteria for a current depressive episode and was excluded from analysis.

Age difference between groups was analyzed by means of a one-way Welch ANOVA and differences in education level and gender between groups were analyzed using Fisher's exact test. Separate mixed ANOVA's with group (CT + tDCS, WMT + sham tDCS and WMT + tDCS) as a between-subject factor and time (pre- and post-manipulation) as within-subject variable were performed on the POMS scores of depression, anger, tension, fatigue and vigor as a dependent variable to check for the effects of the manipulation on mood.

To investigate differences between real tDCS and sham tDCS in the speed of improvement during working memory training a mixed ANOVA with time as a within-subject variable and working memory training group as between-subject variable (with real tDCS and sham tDCS) was conducted.

To test for the effects of the manipulation on working memory a mixed ANOVA with time (pre- and post-manipulation) as a within-subject variable and group (CT + tDCS, WMT + sham tDCS and WMT + tDCS) as between-subject variable was performed on the RSpan scores. For the reaction time analyses of the IST, median scores were used to reduce influence of outliers in the within-subject data (cf. Koster et al. 2013). In accordance with previous research (De Lissnyder et al. 2012b; Koster et al. 2013) both correct and incorrect trials were included in the data-analyses.¹ A block of trials was considered correct if both numbers of the faces in each category were accurate. For the latencies on the IST, we performed a mixed ANOVA with condition (emotion, gender) and switch type (switch, no-switch) as within-subject factors and group (CT + tDCS, WMT + sham tDCS and WMT + tDCS) as a between-subject factor. For significant interactions, follow-up mixed ANOVA's within the significant factors and *t*-tests were performed.

¹ Analysis based on correct trials only did not alter the conclusions.

To test for the effects of the experimental manipulation on momentary rumination, a mixed ANOVA with the beginning and the end of the relax period (pre- and post-relax period) and relax period itself (pre- and post-manipulation) as within-subject variables and group as a between-subject variable was conducted on the MRSI's.

Due to practical limitations there were less participants with HRV data ($n = 19$ per condition). HRV measures were transformed logarithmically to ensure normal distribution. To analyze effects of the manipulation on HRV a mixed ANOVA with time (during the first and second relax period) as a within-subject variable and group as a between-subject variable was performed on RMSSD and HF power. To follow up on significant interactions *t*-tests were performed. Tau correlations were calculated between RMSSD and HF power on the one hand and the RRS and MRSI's on the other hand to check for correlations between HRV and rumination. Since gender differences have been reported in reactive HRV (Smith et al. 1998; Verkuil et al. 2009), analyses were repeated with female participants only.

Results

Characteristics of the Study Population

Age, $F(2,36) = 1.95$, $p = .17$, and education level, $\chi^2(4) = 3.94$, $p = .48$, were not significantly different between groups. Groups did significantly differ in gender, $\chi^2(2) = 10.99$, $p < .01$, due to a coincidental lack of males in the working memory training + sham tDCS group.

Effects of Experimental Manipulation on Mood

Separate mixed ANOVA's with group as a between-subject factor and time (pre- and post-manipulation) as within-subject variable were performed on the POMS scores of depression, anger, tension, fatigue and vigor as a dependent

variable to check for the effects of the manipulation on mood. The interaction effect of Time \times Group did not reach significance in any of the ANOVA's, F 's < 1.26 , suggesting changes in mood are independent of tDCS and working memory training and therefore are unlikely to confound potential effects on cognitive control. Mean (with SD) scores of the subscales of the POMS across groups at two time points (before and after the manipulation) are reported in Table 1.

Speed of Improvement During Working Memory Training

A mixed ANOVA with time as a within-subject variable and working memory training group as a between-subject variable (with real tDCS and sham tDCS) was conducted to explore effects of tDCS on the speed of improvement during the working memory training. This analysis revealed a significant effect of time, $F(18,24) = 2.74$, $p < .05$, $\eta_p^2 = .67$, with a higher n -back at the end of the training ($M = 2.28$, $SD = 0.85$) than at the beginning ($M = 1.60$, $SD = 0.66$). Although there was no significant Time \times Group interaction, $F(18,24) = 1.25$, $p = .30$, there was a marginal significant main effect of group, $F(1,41) = 3.88$, $p = .056$, $\eta_p^2 = .09$, with a higher mean reached n -back for the real tDCS condition ($M = 2.32$, $SD = 0.58$) than for the sham tDCS condition ($M = 2.05$, $SD = 0.27$).

Effects of Experimental Manipulation on RSpan Accuracy

A mixed ANOVA on RSpan score as a dependent variable, time as a within-subject variable and group as between-subject variable revealed a significant main effect of time on working memory accuracy, $F(1,62) = 17.32$, $p < .001$, $\eta_p^2 = .22$, with scores post-manipulation being higher ($M = 21.06$, $SD = 5.69$) than pre-manipulation ($M = 18.58$, $SD = 5.49$). Contrary to our hypothesis, there was no significant main effect of group, $F(2,62) = 1.48$, $p = .24$, nor a significant Time \times Group interaction, $F(2,62) = 1.25$, $p = .29$.

Effects of Experimental Manipulation on IST Switching Latencies

Analyses revealed an average accuracy rate of 90 %. For the latencies on the IST, a mixed ANOVA with condition and switch type as within-subject factors and group as a between-subject factor was performed. This revealed a significant main effect of condition, $F(1,62) = 21.73$, $p < .001$, $\eta_p^2 = .26$, with reaction times in the emotion condition ($M = 1107$ ms, $SD = 240$ ms) being slower than reaction times in the gender condition ($M = 1026$ ms, $SD = 198$ ms). Furthermore, analyses revealed a significant main effect of switch type, $F(1,62) = 391.54$, $p < .001$, $\eta_p^2 = .86$, with reaction times on switches ($M = 1289$ ms, $SD = 284$ ms) being slower than reaction times on no-switches ($M = 862$ ms, $SD = 144$ ms).

More importantly, analyses revealed a significant Switch Type \times Group interaction, $F(2,62) = 3.96$, $p < .05$, $\eta_p^2 = .11$. Comparisons within the switch types showed that there is a significant difference between groups on switches, $F(2,62) = 3.40$, $p < .05$, $\eta_p^2 = .10$. Post-hoc tests revealed this effect was due to the working memory training + sham tDCS group being significantly slower on switches than both the control training + tDCS group, $t(35.11) = 2.10$, $p < .05$, and the working memory training + tDCS group, $t(41) = 2.07$, $p < .05$, see Fig. 2. There was no significant difference in reaction time on switches between the groups with real tDCS, $t(41) = 0.20$, $p = .85$. This difference between groups was not significant in no-switches, $F(2,62) = 1.24$, $p = .30$.

Given the absence of a significant Group \times Condition or Group \times Condition \times Switch type interaction no further analyses were performed to investigate valence-specific effects.

Effects of Experimental Manipulation on State Rumination

To measure momentary ruminative self-referent thinking the MRSI was administered at the beginning and end of a 10 min relax period both before and after experimental

Table 1 Mean (with SD) scores of mood types of the Profile of Mood scales (POMS) before (T1) and after (T2) manipulation

	CT + tDCS		WMT + sham tDCS		WMT + tDCS	
	T1	T2	T1	T2	T1	T2
Depression	1.05 (1.96)	0.27 (0.63)	1.18 (2.65)	0.95 (1.70)	0.38 (0.86)	0.33 (0.66)
Anger	0.64 (0.95)	0.64 (2.56)	0.55 (1.44)	0.36 (0.79)	0.52 (0.68)	1.05 (1.99)
Tension	2.36 (2.80)	0.95 (1.79)	2.23 (2.49)	1.09 (1.69)	2.05 (2.33)	0.90 (1.67)
Fatigue	2.68 (2.63)	2.50 (3.10)	3.23 (2.79)	2.91 (2.29)	2.14 (2.08)	2.86 (3.61)
Vigor	9.00 (2.86)	7.50 (3.84)	11.59 (3.03)	9.77 (4.59)	11.67 (3.14)	9.05 (4.21)

CT control training, WMT working memory training, tDCS transcranial direct current stimulation

manipulation. To explore the effects of the manipulation on momentary rumination, a mixed ANOVA with the beginning and the end of the relax period (pre- and post-relax period) and relax period itself (pre- and post-manipulation) as within-subject variables and group as a between-subject variable was conducted. This revealed a significant main effect of relax period (pre- and post-manipulation), $F(1,62) = 19.33$, $p < .001$, $\eta_p^2 = .24$, with participants ruminating more pre-manipulation ($M = 17.42$, $SD = 6.03$) than post-manipulation ($M = 14.98$, $SD = 6.05$). There was also a significant main effect of the beginning and the end of the relax period, $F(1,62) = 41.06$, $p < .001$, $\eta_p^2 = .40$, with participants ruminating more at the end of a relax period ($M = 18.23$, $SD = 6.53$) than at the beginning ($M = 14.28$, $SD = 5.66$). Furthermore, there was a significant interaction between relax period pre- and post-manipulation and the beginning and end of a relax period, $F(1,62) = 8.02$, $p < .01$, $\eta_p^2 = .12$. This interaction is due to a larger difference between the pre- and post-manipulation scores at the beginning of the relax period (M difference = 3.35, SD difference = 5.21) than at the end of a relax period (M difference = 1.64, SD difference = 4.91). These effects are illustrated in Fig. 3. Contrary to our predictions, there were no significant interactions with group, F 's < 1.

Effects of Experimental Manipulation on Heart Rate Variability

A mixed ANOVA with time (during the relax period pre- and post-manipulation) as a within-subject variable and group as a between-subject variable revealed a significant effect of time on log transformed RMSSD,

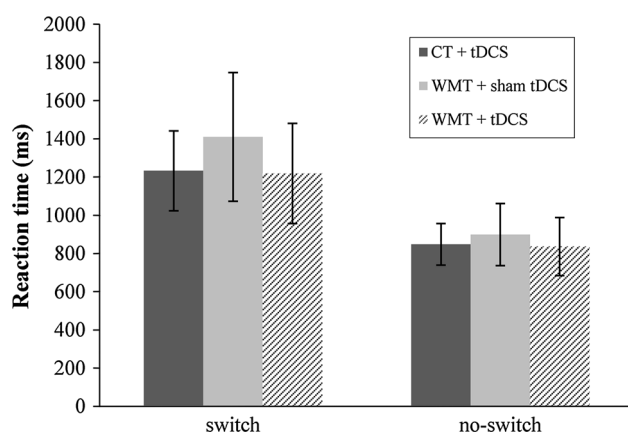


Fig. 2 Reaction time on switches versus no-switches on the internal shift task (IST) after manipulation for the control training (CT) + transcranial direct current stimulation (tDCS) group, the working memory training (WMT) + sham tDCS group and the WMT + tDCS group. $*p < .05$

$F(1,53) = 44.47$, $p < .001$, $\eta_p^2 = .46$. Log transformed RMSSD was higher after manipulation ($M = 1.66$, $SD = 0.18$) than before manipulation ($M = 1.55$, $SD = 0.20$). More importantly, there was a significant interaction between time and group, $F(2,53) = 3.77$, $p < .05$, $\eta_p^2 = .13$. This effect was due to a higher increase in log transformed RMSSD in the working memory training + sham tDCS group ($M = 0.16$, $SD = 0.12$) than the control training + tDCS group ($M = 0.05$, $SD = 0.10$, $t(36) = 2.99$, $p < .01$). The difference between the control training + tDCS group and the working memory training + tDCS group was not significant, $t(35) = 1.43$, $p = .16$, neither was the difference between the working memory training + sham tDCS group and working memory training + tDCS group, $t(35) = 1.19$, $p = .24$.

These effects were replicated with the log transformed HF power measure ($F(1,53) = 21.74$, $p < .001$, $\eta_p^2 = .29$ for time; and $F(2,53) = 5.05$, $p < .05$, $\eta_p^2 = .16$ for the interaction with group). Within the HF power measure, there was a significant difference between the control training + tDCS group and both the working memory training + sham tDCS group, $t(36) = 3.50$, $p < .01$, and the working memory training + tDCS group, $t(35) = 2.07$, $p < .05$ (see Fig. 4). Similar to the RMSSD measure, within the HF power measure the difference between the groups with working memory training was not significant, $t(35) = 0.77$, $p = .45$.

Furthermore, log transformed HRV did not significantly correlate with measures of momentary rumination (MRSI), nor with trait rumination (RRS). Yet, within women all measures of log transformed HRV significantly correlated with the RRS (RMSSD pre-manipulation: $\tau = -.24$, $p < .05$, HF power pre-manipulation: $\tau = -.21$, $p < .05$,

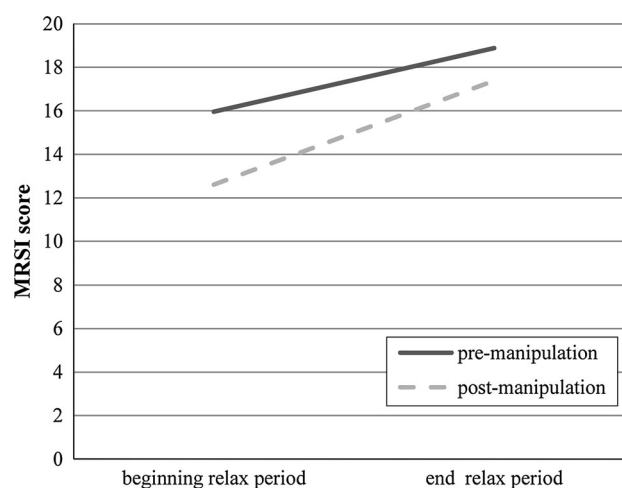


Fig. 3 Change in state rumination assessed by the Momentary Ruminative Self-focus Inventory (MRSI) from the beginning to the end of the relax periods pre- and post-manipulation

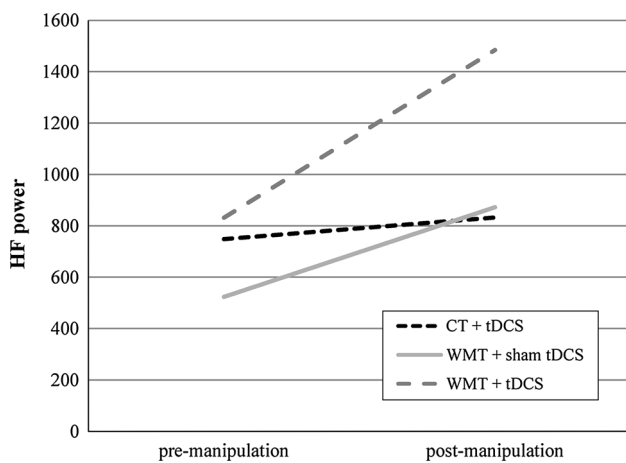


Fig. 4 Change between groups in high frequency (HF) power heart rate variability scores (prior to normalization) from the pre-manipulation to the post-manipulation relax period. The control training (CT) + transcranial direct current stimulation (tDCS) group had a significantly slower increase in heart rate variability than both groups with working memory training (WMT)

RMSSD post-manipulation: $\tau = -.21, p < .05$, HF power post-manipulation: $\tau = -.22, p < .05$).

Discussion

This study investigated the influence of a single session of anodal tDCS applied to the left DLPFC and working memory training on working memory performance and rumination. First of all, there were no direct effects of neuromodulation and working memory training on changes in mood. Therefore, it is unlikely that changes in mood confounded any of the other potential effects on working memory. This allows to examine whether a single session working memory training, either alone or combined with tDCS, can provide transient changes in working memory performance, allowing to test causal influences of working memory on state rumination.

We found mixed effects of the different conditions on working memory tasks. Although, there was a general increase in accuracy scores on the running span task, there were no significant differential effects of tDCS and working memory training on scores of the running span task. The running span is a working memory task that provides information regarding accuracy, but not latencies. A recent meta-analysis of Brunoni and Vanderhasselt (2014) found that anodal tDCS on the DLPFC only improves reaction time in working memory tasks, not accuracy rates. This could explain why we failed to find effects of the experimental manipulation on the running span task. Hence, future research should include working memory tasks that measure reaction times of working memory pre- and post-

tDCS and working memory training, in order to conclude about possible synergistic effects of neuromodulation and working memory training on a non-emotional working memory task.

In contrast, some changes emerged for the IST, which is a working memory task that measures latencies for updating and switching between emotional and non-emotional representations. Participants were overall slower in the emotion condition and on switch trials in the IST. More importantly, both groups with real tDCS were significantly faster than the working memory training + sham tDCS group on trials with switches. This effect suggests that tDCS may improve the speed of switching between types of information in working memory. This effect is not different between participants either receiving an additional working memory training or control training. This finding is in line with the meta-analysis of Brunoni and Vanderhasselt (2014) which summarizes the results of 12 studies on the effects of non-invasive stimulation of the DLPFC on working memory. Brunoni and Vanderhasselt (2014) found that tDCS resulted in an improvement of the speed of working memory. Moreover, during working memory training in the current study there was a marginally significant effect towards an overall higher mean *n*-back for the real tDCS condition compared to the sham tDCS condition. This may suggest a small beneficial effect of tDCS.

Participants reported more ruminative thoughts following the rest period compared to the beginning of the rest period. This conforms that, similar to Marchetti et al. (2013), the resting period was successful in evoking momentary rumination. Furthermore, tDCS and working memory training did not directly influence the occurrence of self-reported ruminative thoughts. In the current study, the groups with working memory training had a significantly higher increase in HRV before compared to after the manipulation than the control training + tDCS group for the HF power measure. For the RMSSD measure only the working memory training + sham tDCS group had a significantly higher increase than the control training + tDCS group. This suggests a beneficial effect of working memory training on HRV and might indicate a subtle increase in adaptive emotion regulation, since HRV provides an index of adaptive self-regulation (for a review Appelhans and Luecken 2006; Thayer et al. 2012). It has already been shown that HRV can influence cognitive performance (Hansen et al. 2003). However, the current study suggests that cognitive training can also influence HRV. HRV did not correlate significantly with measures of self-reported state rumination nor trait rumination. However, in women all measures of HRV during the relax periods before and after manipulation correlated significantly with trait rumination. This is in line with Ottaviani et al. (2009) and Woody et al. (2014). Ottaviani et al. (2009) recruited a

mixed gender sample and found that women showed a greater HRV decrease during rumination than men. Woody et al. (2014) recruited a female sample since women report significant higher levels of rumination and depression than men (Nolen-Hoeksema and Jackson 2001). They found a significant correlation between rumination and HRV. This attests to the validity of using HRV as a physiological marker of rumination.

There are several limitations of the current study. For instance larger effects are to be expected in a sample suffering from elevated rumination. In this regard, recruiting a healthy sample could pose the risk of floor effects in rumination. However we did find elevated state rumination after a 10 min rest period. Therefore it was possible to study whether the increase in rumination after a 10 min rest period declines after receiving tDCS and/or working memory training. Another limitation is the absence of a group with sham tDCS and control training. It was possible to deduce the effects of tDCS from the comparison of the group with sham tDCS and both groups with real tDCS, and the effects of working memory training from the comparison of the group with control training and both groups with working memory training. However in the absence of a group with sham tDCS and control training it was not possible to check whether the effects differ when working memory is not manipulated in any way.

To summarize, this study set out to explore the influence of (combined) neuromodulation and working memory training on working memory performance and rumination. Participants who received real tDCS were significantly faster in switching between information in working memory than participants with sham tDCS. Moreover, participants receiving tDCS performed slightly better during the working memory training. These findings indicate that a single session of tDCS suffices to produce transient changes in non-emotional working memory. In contrast, a single session of working memory training does not suffice to produce transient changes in working memory. Other studies that found improvement of working memory through cognitive training combined with tDCS used at least 5 sessions (Brunoni et al. 2014; Martin et al. 2013; Segrave et al. 2014). However, multiple tDCS sessions on healthy participants might pose ethical challenges, since its effects can last up to 12 months (Dockery et al. 2009). No effect of a single session tDCS nor working memory training was found on self-reported state rumination. However, working memory training did increase HRV, which is an index of adaptive self-regulation (for a review Appelhans and Luecken 2006; Thayer et al. 2012). This suggests that a single session of working memory training might already have subtle beneficial effects on self-regulation.

Thus, a single session working memory training can be used to manipulate the physiological correlate of

rumination, suggesting a causal relationship, and tDCS can be used to manipulate working memory experimentally, in line with the role of the prefrontal cortex in working memory. However, there are no synergistic effects on rumination of this combined procedure in a single session and our results suggest there is only limited value in combining working memory training and tDCS to produce a transient manipulation of working memory.

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Compliance with Ethical Standards

Conflict of Interest Laura M. S. De Putter, Marie-Anne Vanderhasselt, Chris Baeken, Rudi De Raedt and Ernst H. W. Koster declare that they have no conflict of interest.

Ethical Standard The present study was approved by the Medical Ethical Committee of Ghent University Hospital. All procedures followed were in accordance with the ethical standards of this responsible committee on experiments involving human participants.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Animal Rights This article does not contain any studies with animals performed by any of the authors.

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