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Boosting Cognition: Effects of Multiple-Session Transcranial Direct Current Stimulation on Working Memory

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Abstract

■ Transcranial direct current stimulation (tDCS) is a promising tool for neurocognitive enhancement. Several studies have shown that just a single session of tDCS over the left dorsolateral pFC (lDLPFC) can improve the core cognitive function of working memory (WM) in healthy adults. Yet, recent studies combining multiple sessions of anodal tDCS over lDLPFC with verbal WM training did not observe additional benefits of tDCS in subsequent stimulation sessions nor transfer of benefits to novel WM tasks posttraining. Using an enhanced stimulation protocol as well as a design that included a baseline measure each day, the current study aimed to further investigate the effects of multiple sessions of tDCS on WM. Specifically, we investigated the effects of three subsequent days of stimulation with anodal (20 min, 1 mA) versus sham tDCS (1 min, 1 mA) over lDLPFC (with a right supraorbital reference) paired with a challenging verbal WM task. WM performance was measured with a verbal WM updating task (the letter *n*-back) in the stimulation sessions and several WM transfer tasks (different letter set *n*-back, spatial *n*-back, operation span) before and 2 days after stimulation. Anodal tDCS over lDLPFC enhanced WM performance in the first stimulation session, an effect that remained visible 24 hr later. However, no further gains of anodal tDCS were observed in the second and third stimulation sessions, nor did benefits transfer to other WM tasks at the group level. Yet, interestingly, post hoc individual difference analyses revealed that in the anodal stimulation group the extent of change in WM performance on the first day of stimulation predicted pre to post changes on both the verbal and the spatial transfer task. Notably, this relationship was not observed in the sham group. Performance of two individuals worsened during anodal stimulation and on the transfer tasks. Together, these findings suggest that repeated anodal tDCS over lDLPFC combined with a challenging WM task may be an effective method to enhance domain-independent WM functioning in some individuals, but not others, or can even impair WM. They thus call for a thorough investigation into individual differences in tDCS respondence as well as further research into the design of multisession tDCS protocols that may be optimal for boosting cognition across a wide range of individuals. ■

INTRODUCTION

Transcranial direct current stimulation (tDCS) is a safe and noninvasive brain stimulation method in which a low-voltage electric current (≤2 mA) is run between two scalp electrodes: the anode (the positive electrode) and cathode (negative electrode). By modulating the membrane potential of underlying cortical neurons, tDCS may alter brain functioning. More specifically, stimulation with tDCS may temporarily make neurons more (anodal; facilitating) or less (cathodal; inhibiting) prone to fire action potentials (Kuo & Nitsche, 2012; Nitsche et al., 2008).

Working memory (WM) is considered a core cognitive function underlying performance in many everyday life situations as it allows us to retain and monitor information over brief periods of time (Baddeley, Sala, Robbins, & Baddeley, 1996). As WM may be disturbed in psychiatric conditions such as schizophrenia (Barch & Ceaser, 2012) and decrease in older age, there is a growing interest in methods to enhance WM functioning, for example, with intensive computerized task training. Although initial results of WM training studies suggested widespread cognitive benefits (Chein & Morrison, 2010; Klingberg, 2010; Jaeggi, Buschkuehl, Jonides, & Perrig, 2008), more recent, well-controlled studies found only limited transfer of improvements after WM training (Harrison et al., 2013; Redick et al., 2013). Together with long training times (typically >20 hr), this substantially limits the practical value of WM training as method to improve cognitive functioning.

Interestingly, a decade ago a pioneering study by Fregni and colleagues (2005) reported that a single session of anodal tDCS (vs. sham stimulation) over the left dorsolateral pFC (lDLPFC) could improve verbal WM in healthy adults. This finding has been replicated and extended to a variety of populations (Hill, Fitzgerald, & Hoy, 2016; Mancuso, Ilieva, Hamilton, & Farah, 2016; Bennabi et al., 2014; but also see Brunoni & Vanderhasselt, 2014), providing substantial support for the claim that directly modulating the brain with anodal lDLPFC stimulation may be a promising new tool for neurocognitive enhancement in healthy as well as clinical populations.

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Moreover, the effects of tDCS on behavior do not seem to be limited to temporary changes in excitability only but may involve actual longer-lasting neuroplastic changes. This may make tDCS a specifically useful method for enhancing learning. Indeed, anodal tDCS over relevant areas may speed up the effects of behavioral motor and visuomotor revalidation training after stroke (Hashemirad, Zoghi, Fitzgerald, & Jaberzadeh, 2016). For example, in one study, 3 months of visual field training combined with tDCS resulted in improvements typically observed after 6 months of behavioral training only (Plow, Obretenova, Fregni, Pascual-Leone, & Merabet, 2012). Similar effects have been found in healthy individuals and in the cognitive domain, where repeated tDCS has been shown to facilitate artificial number learning (Cohen Kadosh, Soskic, Iuculano, Kanai, & Walsh, 2010; with effects still apparent 6 months later) and response inhibition training (Ditye, Jacobson, Walsh, & Lavidor, 2012). Together, these findings raise the premise that anodal tDCS over lDLPFC paired with WM training may speed-up and/or strengthen WM training effects.

Although many studies have reported effects of single-session tDCS, so far only three studies have examined the effects of multiple sessions of anodal lDLPFC stimulation and verbal WM training on WM in healthy adults. First, Lally, Nord, Walsh, and Roiser (2013) found no additional improvement on a verbal WM task during anodal versus sham stimulation over the course of two sessions (although a post hoc analysis did show larger enhancements in the anodal group on the first day). Second, Martin et al. (2013) also found no differences between an anodal and sham group in a study with 10 sessions of combined tDCS and verbal WM training, not on the trained task itself (when group baseline performance differences were taken into account) nor on other cognitive tasks administered in a separate session 1 day after stimulation to assess possible transfer of training benefits. Third, with a similar design, Richmond, Wolk, Chein, and Olson (2014) did find a larger increase in verbal WM performance over 10 training sessions in the anodal compared with the sham tDCS group. Yet, they too failed to observe larger posttraining improvements on additional cognitive transfer tasks. Collectively, these initial findings thus provide little support for the notion that multiple sessions with anodal lDLPFC tDCS and verbal WM training may lead to larger persistent and transferable WM improvements than WM training alone.

However, these null findings may be a consequence of particular design choices in these studies. In tDCS research, several parameters are pivotal in determining its effects, including electrode location, stimulation intensity and duration, and the task paired with stimulation. In the three studies described above, we believe that some of these parameters may not have been optimal for inducing verbal WM enhancements.

First and perhaps most importantly, electrode location critically determines current flow through the brain and thereby the precise cortical regions that are affected (see Nitsche et al., 2008). Notably, all above studies used a different setup than the single-session studies that found WM improvements, with the anode (i.e., the active electrode) over lDLPFC (electrode site F3) and the cathode (i.e., the reference) over the right OFC (rOFC; i.e., the contralateral forehead). All three studies placed the anode over lDLPFC, but the cathode was placed differently. Both Lally et al. (2013) and Martin et al. (2013) chose extraencephalic references with the cathode on the contralateral cheek and shoulder, respectively. Although common in, for example, the motor domain, it is conceivable that with this setup more medial parts of lDLPFC that are also important for WM are missed. Moreover, Richmond et al. (2014) placed the cathode over the right DLPFC (F4), a region known to be involved in WM (Au et al., 2016; Berryhill & Jones, 2012; Owen, McMillan, Laird, & Bullmore, 2005). The possible inhibitory effect of the cathode over this region may make this electrode setup suboptimal for inducing WM improvements.

Two other parameters that play an important role in the effect of tDCS on behavior are stimulation intensity and duration. Most effective single-session tDCS studies used a stimulation strength of 1 mA to boost WM in healthy adults (Andrews, Hoy, Enticott, Daskalakis, & Fitzgerald, 2011; Mulquiney, Hoy, Daskalakis, & Fitzgerald, 2011; Ohn et al., 2008; Fregni et al., 2005). Notably, in contrast to the intuitive notion that higher intensities lead to stronger effects, a recent study showed that 1-mA, and not 2-mA, stimulation resulted in the most pronounced WM improvements (Hoy et al., 2013; but see also Teo, Hoy, Daskalakis, & Fitzgerald, 2011). The current strengths of 1.5 and 2.0 mA used by Richmond et al. (2014) and Martin et al. (2013) may thus have been suboptimal. Similarly, longer stimulation durations may not always result in larger effects either. In fact, in the motor domain, longer stimulation times have shown to diminish and sometimes actually result in opposite effects in behavior (see Nitsche et al., 2008). In particular, the 30-min stimulation by Martin et al. (2013) is relatively long compared with the 10–20 min typically used in the single-session literature (and the 10 and 15 min used by Lally et al., 2013; Richmond et al., 2014), which may have reduced its effectiveness.

Finally, the "state" of the stimulated area (i.e., what a participant is doing) may be critical in determining stimulation effects on behavior. Anodal tDCS admitted concurrent with a task (online stimulation) has been shown to be more effective in boosting WM than tDCS admitted during rest (offline stimulation; Mancuso et al., 2016; Martin, Liu, Alonzo, Green, & Loo, 2014; Andrews et al., 2011). Possibly, this is because the targeted brain networks are already engaged in the to-be-modulated cognitive activity. This likely also applies to repeated stimulation. Both Lally et al. (2013) and Martin et al. (2013) used online stimulation. However, in Richmond et al. (2014), the task was only paired with tDCS during the last 5 min of stimulation, and the remainder of the task was done without stimulation, conceivably reducing tDCS effectiveness in enhancing WM.

Adding to the literature in this field, the current study aimed to evaluate the effects of multiple-session lDLPFC stimulation on WM using a setup that may maximize tDCS effectiveness. Similar to single-session verbal WM enhancement studies, the anode was placed over lDLPFC (F3) and the cathode over rOFC (contralateral above the right eye) and stimulation was applied at 1-mA intensity for 20 min. Furthermore, stimulation was paired with a highly demanding verbal WM task (three- and four-letter *n*-back task), as this may be critical for enhancing cognitive functioning (Gill, Shah-Basak, & Hamilton, 2015). The study was conducted using a randomized double-blind design in which participants underwent either active tDCS or sham stimulation (1 min of stimulation) on three consecutive days. The verbal WM task on these days was split in four equal blocks, and stimulation was always applied during the second block. This design allowed us to look at the effects of tDCS at different time windows during and after stimulation. Moreover, for each session, the effects of tDCS on behavior could be contrasted to the first, baseline block of that day, which also permitted us to separate within-session effects of tDCS from between-session carryover effects of previous stimulation.

Furthermore, to determine if our combined tDCS and WM protocol could induce more general WM enhancements, we assessed transfer of potential benefits to different stimuli and task contexts. To this end, before the first and 2 days after the last stimulation session, participants performed three other WM tasks, namely, the same verbal WM task with a different letter set, a spatial version of this task (spatial *n*-back), and a complex span task (the automated operation span [Ospan] task; Unsworth, Heitz, Schrock, & Engle, 2005).

We predicted that, using our optimized stimulation protocol, three daily sessions with anodal versus sham tDCS would first of all result in greater cumulative improvements in verbal WM in the stimulation sessions. Second, we expected tDCS effects to outlast the stimulation and remain apparent 24 hr later, that is, in the baseline blocks of the next day. Third, we expected anodal (vs. sham) tDCS combined with WM practice to induce general WM improvements, as reflected in larger performance improvements on the WM transfer tasks posttraining.

Recently, a growing number of studies have reported that the effect of tDCS may vary substantially across individuals. This may be due to differences in, for example, brain anatomy (Opitz, Paulus, Will, Antunes, & Thielscher, 2015; Kim et al., 2014), baseline performance (Learmonth, Thut, Benwell, & Harvey, 2015; London & Slagter, 2015; Meiron & Lavidor, 2013; Berryhill & Jones, 2012), and/or differences in cortical excitability (Krause & Cohen Kadosh, 2014). Consequently, in standardized tDCS protocols, some individuals may benefit more than others. Therefore, in addition to group level analyses, we post hoc also explored if, across participants, protocol effectiveness (i.e., the extent to which WM was improved in the stimulation sessions) could predict transfer to the WM tasks posttraining. Our final, fourth prediction was that such transfer of benefits should be most apparent in those individuals whose performance increased most in the tDCS combined with WM training sessions.

METHODS

Participants

Fifty participants were recruited via the University of Amsterdam and were compensated for their participation with money or research credits. Participants gave written informed consent before the experiment, as approved by the local ethics committee. All reported no history of psychiatric conditions and had normal or corrected-to-normal vision. They were checked for tDCS contraindications, such as metal implants and sensitive skin (see Nitsche et al., 2008). Furthermore, pilot analyses showed that participants that already started out with high WM accuracy scores in the prestimulation session tended to reach almost perfect performance in the second or beginning of the third stimulation session. To ensure sensitivity to improvements throughout all three stimulation sessions for all participants, we excluded high performers in the prestimulation session from further participation. To determine this, we calculated accuracy scores (in the form of A', see below) over the verbal WM task (Levels 3 and 4 only) during this initial session and excluded participants who showed A' values above 0.90 (n = 15). This threshold corresponded to an average hit rate of 0.85 in the excluded participants. Four participants could not complete the study because of personal or health reasons unrelated to the study. One last participant was excluded because of very poor performance on the verbal WM task throughout the whole experiment (>3 *SD*s below the mean). As a result, 30 participants were left for analysis (anodal group: 4 men, 11 women, mean age = 21.9 years, *SD* = 2.8; sham group: 5 men, 10 women, mean age = 22.1 years, *SD* = 2.3).

Design and Procedure

Participants came to the lab at the same time each day for a total of five sessions: a first behavioral session (presession), three consecutive days of tDCS stimulation combined with a verbal WM task (stimulation sessions), and a second behavioral session (postsession). Participants were pseudorandomly divided over the two stimulation groups: active versus sham (double-blind between-subject design), matching the groups on gender, age, and WM performance in the presession. In the stimulation sessions, participants received either active (1 mA, 20 min) or sham (1 mA, 1 min) anodal tDCS over the left DLPFC while performing a verbal WM updating task. Moreover, in the

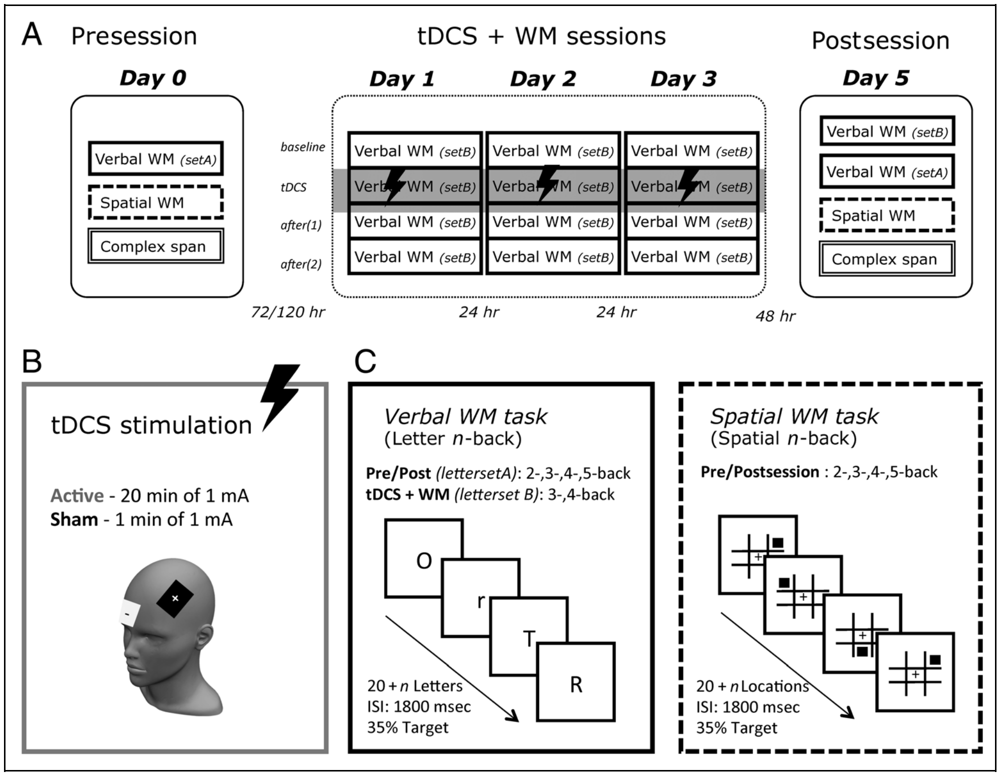

Figure 1. Procedure and tasks. (A) We investigated the effects of three sessions of anodal (vs. sham) lDLPFC tDCS combined with a verbal WM task (a letter *n*-back) on performance on this task, as well as on three WM transfer tasks in a postsession versus presession, namely the same verbal WM task with a different stimulus set, a spatial WM task, and a complex WM span task (the automated Ospan; Unsworth et al., 2005). Order of the verbal and spatial WM transfer tasks was counterbalanced across participants, whereas the complex WM task was always performed last. (B) The active stimulation group received 20 min of 1-mA tDCS, whereas the sham group received only 1 min of stimulation. The anode was always placed on the lDLPFC (F3), and the cathode on the rSOF (above the right eye). (C) In the verbal WM task, a stream of letters was presented, and participants were required to press a button if the current letter was the same as *n* stimuli before. In the spatial version of the task, the stimulus to respond to was a blue square that was presented in one of the eight outer locations of a 3 × 3 grid. In the stimulation sessions, level of *n* in the verbal WM task alternated between 3 and 4 to ensure a challenging task over all three sessions. In the transfer verbal and spatial WM tasks, level of *n* ranged from 2 to 5 to index a broader range of participants' abilities.

separate behavioral pre- and postsession (48 hr after), participants performed three additional tasks: the same verbal WM task with a different stimulus set, a spatial version of the same WM task, and a complex WM span task. As research in animals has indicated that cellular changes induced directly after tDCS have fully returned to baseline levels after 48 hr (see Nitsche et al., 2008), a 2-day gap was implemented between the last stimulation session and the postsession to ensure that the temporary effects of tDCS would have worn out. Please see Figure 1A for a graphical rendering of the different experimental sessions.

Transcranial Direct Current Stimulation

Stimulation was delivered with a battery-driven Eldith DC-stimulator (NeuroConn GmbH, Germany) using two 7 × 5 cm conductive electrodes. Electrodes were placed in saline-soaked sponges and held in place with rubber bands. The anodal electrode was always placed over the left DLPFC (F3 in the 10/20 system), whereas the cathodal electrode was placed over the right supra-orbitofrontal region (centered above the right eye pupil), see Figure 1B. This electrode arrangement is known to result in significant WM enhancements in single stimulation sessions (Andrews et al., 2011; Mulquiney et al., 2011; Teo et al., 2011; Fregni et al., 2005). In each participant, in the first session, the position of F3 was localized using an EEG cap (64 channels, Biosemi, Amsterdam, The Netherlands) and marked on the scalp to ensure the same electrode placement on the subsequent days. Participants in the active stimulation group received 20 min of 1-mA anodal stimulation, whereas those in the sham group received only 1 min of 1-mA anodal stimulation each stimulation session. To reduce discomfort and improve our shamming procedure, in both conditions, the current was ramped up over 30 sec and down over 60 sec. Both participant and experimenter were blind to experimental group and thus to which type of stimulation was applied.

Stimulation Sessions: tDCS + Verbal WM Task

We investigated the immediate effects of anodal versus sham tDCS over left DLPFC on verbal WM across three daily sessions of stimulation. Similar to previous single-session studies (e.g., Fregni et al., 2005), participants performed a letter version of the *n*-back task to probe verbal WM. In this task, participants are presented with a stream of letters, and they have to indicate if the currently presented stimulus is the same as the one presented *n* stimuli back. *n* is an integer, and the value of *n* determines the difficulty level of the task, with higher levels of *n* corresponding to higher WM loads, as more stimuli have to be held in WM in sequential order. A recent study found that anodal (vs. sham) tDCS to lDLPFC only improved poststimulation performance on an attention task when combined with a challenging 3-back but not an easy 1-backverbal WM task (Gill et al., 2015). Therefore, to ensure a challenging task during all stimulation sessions, the level of n used alternated between 3 and 4.

While seated in a comfortable chair behind a computer screen (approximately 90-cm distance), participants first practiced the task before actual data collection started. Stimuli were presented using Presentation software (Neurobehavioral Systems, Inc., Berkeley, CA). The task was divided into four blocks of about 15 min (nonstimulation blocks) or 20 min (stimulation blocks) each, and stimulation was always applied concurrent with the second block of the task (see Figure 1).

Each day, the first block of the task thus served as a baseline, which allowed us to investigate possible carryover effects of tDCS to the next day as well as provided a more accurate measure of within session effects in each session. After this first 15-min block of the task, tDCS was administered for 20 min. To allow itching sensations in the first minute of tDCS stimulation to wear off, the task was started 2 min after the onset of stimulation. The last 5 min of behavioral data during the stimulation block was discarded in the analyses to ensure comparison of blocks of equal length. Our design thus allowed us to compare participants' verbal WM performance before, during, and in two blocks after either active or sham stimulation over left DLPFC.

Each 15-min block of the task consisted of 24 so-called runs, in which level of n alternated every three runs between 3 and 4. Runs consisted of a stream of $20 + n$ stimuli each and were self-paced to allow the participant to take small breaks in between runs. Letters were presented in black (Arial, font size 72) at the center of a white screen for 300 msec each, followed by a 1500-msec ISI in which a fixation cross (Arial, font size 20) was displayed centrally (see Figure 1C). Of the presented letters, 35% were so-called targets, that is, the letter that was the same as the letter presented n letters back. If presented with a target letter, participants were required to respond by pressing the space bar on the keyboard. Two letter sets were used in the experiment, namely [a, b, c, d, e, f, g, h, j, k] and [k, m, n, o, p, r, s, t, u, w]. One of these was always used in the verbal WM in the stimulation sessions, whereas the other letter set was used in the verbal WM transfer task in the pre- and postsession. Letter set assignment was counterbalanced across participants. Furthermore, to prevent the use of a simple visual feature matching strategy by participants, letters could be presented in upper or lower case and still would classify as the same letter (i.e., a target).

Each stimulation session started and ended with filling out questionnaires to assess possible side effects of stimulation on mood and arousal, and physical sensations. To assess mood and arousal levels, a Dutch translation of the short version of the Activation Deactivation Adjective Checklist (AD ACL) was used (Thayer, 1978), that asked participants to respond to 20 items using a 4-point rating scale (namely "definitely feel," "feel slightly," "do not really feel," and "definitely do not feel"). Answers are scored on four subscales: energy (general activation), tiredness (general deactivation), tension (high preparatory arousal), and calmness (low preparatory arousal). The AD ACL has proven reliable and valid, showing high test–retest reliability for each of its subscales (all >.79; Thayer, 1978). The AD ACL was filled out twice each session, and changes in mood and arousal were calculated. In addition, to investigate possible physical side effects of the tDCS stimulation, at the end of the session participants were asked to rate their experience on a 5-item scale (namely "not," "a little," "somewhat," "strongly," and "very strongly") with each of eight following sensations: itching, prickling, burning, pain, headache, fatigue, dizziness, and nausea.

Pre- and Postsession: WM Transfer Tasks

To investigate whether possible verbal WM enhancements after tDCS may reflect more general WM learning, before and 2 days after the three stimulation sessions, subjects participated in a behavioral session in which they performed three WM transfer tasks: the same verbal WM task but with a different letter set (i.e., a new stimulus set), a spatial WM task (i.e., a different domain), and a complex WM span (i.e., a different task).

The presession and postsession were identical, except that the presession ended with brief trial tDCS (30 sec of stimulation) to familiarize the participant with the sensation of tDCS whereas the postsession started with a block of the verbal WM task used the stimulation sessions. Adding this last baseline block allowed us to determine if any tDCS effects observed in the stimulation sessions were still measurable 2 days later. Before the actual task started, participants received instructions and performed a series of practice trials with feedback. Order of the verbal and spatial WM task was counterbalanced between participants. The complex WM span was always performed last, as this task includes feedback about performance and may thus possibly lead to motivational differences between participants.

The verbal WM task in the pre- and postsession was very similar to the verbal WM task of the stimulation sessions. However, to investigate possible transfer to a different stimulus set (i.e., stimulus independent learning), the other letter set was used. Also, level of n in the pre- and postsession ranged from 2 to 5 to index a broader range of participants' abilities. The task started with n level 2 and progressed to n level 5 twice, leading to 48 runs of the task in total.

The spatial WM task was a spatial version of the letter n-back task, with the same task structure and stimulus timing. This task was administered to determine possible transfer of tDCS-induced learning effects to a different domain, namely spatial WM. The stimuli in this task were blue squares (80 by 80 pixels) that could be presented in one of eight outer locations of a 3 × 3 grid (200 by 200 pixels, on a 23-in. LCD monitor with the screen set to 1280 by 1024). Please see Figure 1C for a graphical rendering of the task. As in the pre- and postsession verbal WM task, level of n ranged from 2 to 5 in two sequences, again leading to a total of 48 runs of the task.

The complex span task that we administered was the automated version of the Ospan task (Unsworth et al., 2005) using E-Prime (Psychology Software Tools, Inc., Pittsburgh, PA). In this task, participants are also required to remember sequences of letters, but now in between the presentation of each of these letters, they have to evaluate mathematical equations (75 in total). After three to seven letters and math equations, participants are required to report the letters in the order in which they were presented. To account for individual differences in mathematical solving speed, a maximal response time is determined based on participants' performance on 10 practice operations. To prevent problematic short maximal RTs in the second time the task was administered (i.e., in the postsession) because of familiarity with these practice operations, we composed 10 novel operations of similar difficulty level. Order of the two practice sets was counterbalanced across participants. Please see Unsworth et al. (2005) for further details of the task and stimulus structure of the Ospan.

Data Analysis

Questionnaires

We first examined using the debriefing questionnaires if there were differences between the active stimulation group and sham stimulation group in the number of participants who believed to belong to the active stimulation group using a χ^2 test. To examine whether there were systematical differences in physical sensations between groups, repeated-measures ANOVAs were conducted for each of the eight items on the tDCS side-effects questionnaire with Stimulation Session as a within-subject factor and Group as a between-subject factor. To determine whether there was a difference in the effects of anodal versus sham stimulation on arousal states, scores on each of the four subscales of the AD ACL questionnaire were calculated before and after stimulation for each stimulation session separately and subsequently subtracted from each other to obtain a measure of the effect of electrical stimulation. For each subscale separately, a repeated-measures ANOVA was then conducted, comparing changes in the resulting difference scores across sessions between the groups. A Bonferroni correction was applied to account for multiple comparisons for both questionnaires separately, resulting in an alpha of .05/8 = .0063 for the tDCS side effects questionnaire and an alpha of .05/4 = .0125 for the Short Form AD ACL questionnaire.

WM Performance

For the verbal and spatial n-back tasks, accuracy was operationalized using A'. A' is the nonparametric variant of signal detection theory's d' and takes into account both hits (correct responses) and false alarms (incorrect responses). We reverted to A' because, in our data, we encountered blocks of the task in which participants did not have any false alarms, and d' cannot account for these situations. A' can be calculated from hit rate (H) and false alarm rate (F) with the following formula (Zhang & Mueller, 2005):

$$A' = \begin{cases} \frac{3}{4} + \frac{H-F}{4} - F(1-H) & \text{if } F \le 0.5 \le H \\ \frac{3}{4} + \frac{H-F}{4} - \frac{F}{4H} & \text{if } F \le H < 0.5 \\ \frac{3}{4} + \frac{H-F}{4} - \frac{1-H}{4(1-F)} & \text{if } 0.5 < F \le H \end{cases}$$

A' scores range from 0 to 1, in which 0 indicates *chance performance* and 1 indicates *perfect accuracy*. To determine effects of stimulation on response speed and check whether any changes in accuracy may be explained by altered speed accuracy trade-offs, we also computed average RTs using correct response trials only.

For the Ospan task, we used the so-called Total Score as our primary measure of WM functioning. Total Score is calculated as the sum of all the letters that were recalled in the correct order. Also, we looked at mathematical operations errors (math errors) to check for possible trade-offs between letter memory and math performance.

Analytical Approach

To test our first prediction that anodal tDCS would produce larger gains on the WM task than sham stimulation in the three stimulation sessions, we ran a mixed 3 × 4 × 2 repeated-measures ANOVA for A' or RT separately with Session (Day 1, Day 2, Day 3) and Block (before, during tDCS, after(1), after(2)) as within-subject factors and Group (active vs. sham) as a between-subject factor. As we did not have hypotheses for differential effects of stimulation on difficulty levels, accuracy and RT data were collapsed over levels of n.

Furthermore, to test our second prediction that potential effects of tDCS on verbal WM performance would remain apparent 24 (or 48) hr after stimulation, we conducted a 4 × 2 ANOVA on A' of the baseline blocks with Session (Day 1, Day 2, Day 3, and Day 5) as a within-subject factor and Group as a between-subject factor. Whenever an interaction with Group was observed, follow-up tests were run to determine if effects could be ascribed to active or sham stimulation. Similarly, whenever an interaction with Session was observed, we ran additional analyses to investigate the exact time course of stimulation effects.

Additionally, to examine if in the stimulation sessions, physical sensations differed between the active and sham tDCS conditions, we ran a 3 × 2 mixed ANOVA for each of the eight sensations on the tDCS side-effects questionnaire, with Session as a within-subject and Group as a between factor. To account for multiple comparisons, a Bonferroni correction was applied, leading to an alpha of .05/8 = .0063. Similarly, to assess whether mood and/or arousal were differentially affected by stimulation, we ran a 3 × 2 mixed ANOVA separately for each of the four subscales of the short form AD ACL, with Session as within-subject factor and Group as a between-subject factor. Bonferroni correction led to an alpha of .05/4 = .0125 for the AD ACL questionnaire.

Our third prediction was that anodal stimulation combined with WM training would induce general improvements in verbal WM performance, that is, that are not specific to the particular stimuli, domain, and task paired with stimulation. To this end, we ran mixed 2 × 4 × 2 repeated-measures ANOVAs separately on the A' and RT data from the verbal and spatial WM transfer tasks with Session (Day 0 and Day 5) and level of n (2, 3, 4, 5) as within-subject factors and Group as a between-subject factor. Additionally, we analyzed the pre- and postsession performance scores for the verbal and spatial WM task for each level of n separately, as we hypothesized that learning effects may be more pronounced at higher levels of difficulty, where there may be more room for improvement. Finally, to investigate transfer of possible learning effects to performance on the Ospan task, we ran a 2 × 2 ANOVA on Total score and Math errors with the within-subject factor Session and between-subject factor Stimulation Group.

To test our fourth, final prediction that transfer of benefits may be more pronounced in individuals who displayed the largest improvements in the stimulation sessions, we ran cross-subject Spearman correlations (two-tailed) between tDCS-induced changes in WM performance in the stimulation sessions and changes in performance on the transfer WM tasks, separately for the sham and anodal stimulation groups.

All statistical analyses were conducted using the Statistical Package for the Social Sciences for Mac OS, Version 20 (IBM, Armonk, NY). In case of significant main or interaction effects, post hoc analyses were performed to further clarify the results when suitable. Whenever appropriate, Greenhouse–Geisser-corrected results are reported.

RESULTS

Questionnaires

All participants tolerated the tDCS well. Moreover, debriefing questionnaires showed that the majority of participants in both groups believed to belong to the active stimulation group (active group, 57.1% and sham, 64.3%; $\chi^2(1) = 0.15$, $p = .70$), indicating that our sham control procedure was successful.

On the tDCS side effects questionnaire, the active stimulation group reported slightly higher feelings of itching ($F(1, 27) = 4.27$, $p = .049$) and prickling ($F(1, 27) = 5.63$, $p = .025$) than the sham group. Also, over the sessions, both groups reported higher levels of headache (main effect Session, $F(2, 54) = 3.45$, $p = .039$) and fatigue (main effect Session, $F(2, 54) = 4.09$, $p = .022$). However, these effects did not remain significant after correction for multiple comparisons (all other ps > .061). Thus, the stimulation groups did not significantly differ in reported levels of physical sensations in the stimulation sessions.

The AD ACL questionnaire revealed a main effect of Session on the Energy subscale ($F(2, 52) = 6.01$, $p = .008$), albeit no Group × Session ($F(2, 52) = 0.67$, $p = .49$), reflecting that both groups of participants felt less energetic at the end of the first and second stimulation session compared with the third. For the subscale Tiredness, the main effect of Session almost reached significance after correction for multiple comparisons ($F(2, 50) = 4.58$, $p = .015$) but reported tiredness also did not differ between groups (Group × Session $F(2, 50) = 0.67$, $p = .49$). Although participants in the sham group showed a small drop on the subscale Tension (mean = −0.615, SE = 0.274) while the active group did not (mean = 0.143, SE = 0.226), this difference ($F(1, 25) = 4.61$, $p = .042$) did not survive the Bonferroni correction. None of the other main effects or interactions reached significance (all ps > .058), thus indicating that our active and sham stimulation did not exert differential effects on mood and arousal in our participants.

Stimulation Sessions: Immediate Effects of Multiple-session tDCS on Verbal WM

We tested our first prediction that multiple sessions with prefrontal anodal stimulation would lead to cumulative verbal WM enhancements by examining the effects of anodal versus sham tDCS on accuracy and RTs on the verbal WM task in the stimulation sessions.

Accuracy

Accuracy on the verbal WM task improved over the three stimulation sessions in both the active and sham stimulation groups (main effect Session, $F(2, 56) = 25.89$, $p < .001$). However, whereas the active group shows a specific rapid improvement in the first stimulation session, the sham group displays a more gradual improvement in A' over all three sessions (see Figure 2). This pattern was captured by a significant three-way interaction between Session × Blocks × Stimulation condition ($F(6, 168) = 3.53$, $p = .017$). The overall ANOVA further showed a significant interaction between Session × Blocks ($F(6, 168) = 4.33$, $p = .006$). The main effect of Stimulation Group ($p = .67$) and all other interactions were not significant (all ps > .48).

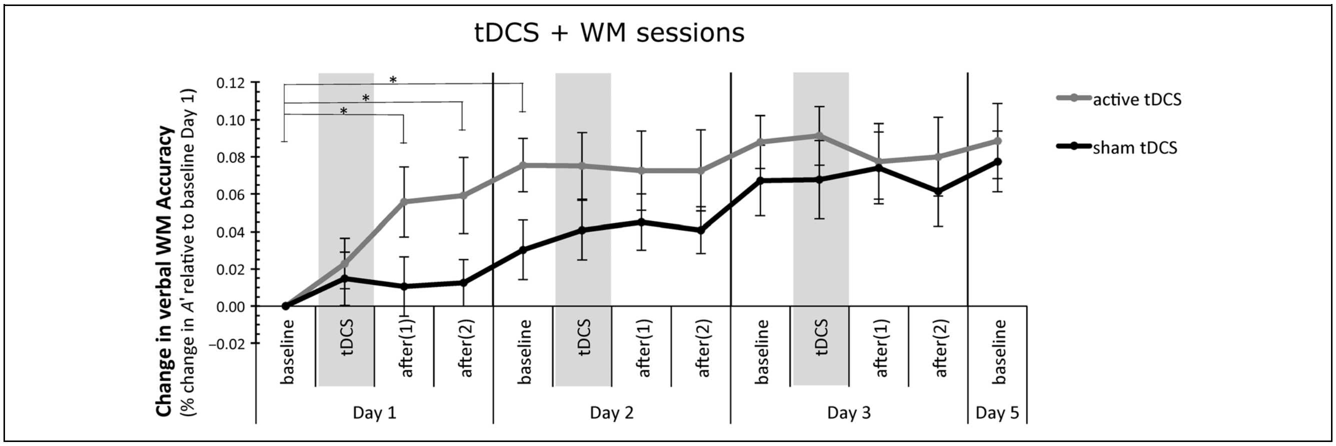

Figure 2. Immediate effects of active versus sham stimulation on the verbal WM task in the combined tDCS and WM sessions. Anodal tDCS over lDLPFC increased accuracy only on the first day of stimulation. Improvements were significant for the two blocks after stimulation ended and remained apparent 24 hr after. No effect of active versus sham tDCS on WM was found in the second and third day of stimulation. (Note that results are displayed here as percent change from baseline, as groups did not differ in baseline performance. Statistical analyses were done on the actual A' values.)

Additional post hoc repeated-measures ANOVAs for each session separately confirmed that tDCS improved WM only in the initial stimulation session (interaction between Group × Block, $F(3, 84) = 3.20$, $p = .047$), but not in the subsequent second ($p = .427$) or third session ($p = .409$). Notably, performance was not yet at ceiling level in these sessions (mean A' second session for the active group was 0.89 and sham was 0.89; mean A' third session active group was 0.90 and sham was 0.91), indicating that this effect cannot simply be explained by lack of room for further improvement (mean A' first session active is 0.86 and sham is 0.87).

To determine if the difference between groups in change in WM performance in the first session was driven by changes in the active stimulation group, as one would expect, we conducted further follow-up analyses separately per stimulation group. These confirmed that participants in the active group significantly improved over blocks in the first session ($F(3, 42) = 6.75$, $p = .005$), whereas the sham stimulation group did not ($F(3, 42) = 0.68$, $p = .50$). Moreover, planned contrasts in the active group showed that this main effect of block in particular reflected a significance increase in accuracy after stimulation ended, as indicated by significant higher accuracy in the first ($t(14) = 2.96$, $p = .011$) and second block ($t(14) = 2.96$, $p = .010$) after stimulation compared with baseline. Performance in the block during stimulation differed from baseline at trend level ($t(14) = 1.80$, $p = .094$). An independent t test between the active and sham groups in the baseline block in the first stimulation session showed no significant difference in WM performance ($t(28) = 1.14$, $p = .26$), indicating that the groups did not differ in performance before tDCS was applied.

To investigate our second prediction that the immediate effects of tDCS should still be present 24–48 hr after stimulation, we compared accuracy in the baseline blocks of the three stimulation sessions and the postsession. The active and sham groups showed similar improvements over sessions (main effect Session, $F(3, 84) = 31.90$, $p < .001$; main effect Stimulation Group, $p = .53$; Session × Group, $p = .13$). However, as the effect of active tDCS seemed to be specific to the first stimulation session, we post hoc also compared baseline performance between groups in the first and second stimulation session only. This revealed a larger improvement in the active stimulation group than in the sham stimulation group (Session × Group, $F(1, 28) = 4.99$, $p = .034$), indicating that the stimulation effects observed in the first session may have carried over to the next day.

RTs

Next to accuracy, we also examined if anodal stimulation combined with WM training speeded up RTs on the WM task. Participants in both groups became faster both within each (main effect Blocks, $F(3, 84) = 14.20$, $p < .001$) and across the three stimulation sessions (main effect Session, $F(2, 56) = 15.24$, $p < .001$). However, this reduction was the same in the active and sham stimulation groups (Session × Group, $F(2, 56) = 0.20$, $p = .76$). The main effect of Stimulation Group ($p = .67$) and all other interaction effects were not significant (all ps $> .21$). Thus, anodal tDCS did not affect response speed on the verbal WM task in the stimulation sessions.

To summarize, partially in line with our first prediction, anodal tDCS improved verbal WM accuracy in the first, but not in the second and third, stimulation session. As tDCS did not affect RTs, these effects cannot simply be explained as a speed–accuracy trade-off nor did we observe differential levels on physical, mood, and arousal scales. Furthermore, partially in line with our second prediction, the effects of tDCS were visible in both blocks after stimulation and remained apparent 24 hr after the first stimulation session.

Pre- and Postsession: Transfer Effects of Combined tDCS and Verbal WM Practice

Next, to test our third prediction, we examined if the observed improvements in verbal WM performance by anodal tDCS in the stimulation sessions may reflect more general WM learning effects. To this end, we investigated differences between the active and sham stimulation groups in performance on the three transfer tasks administered in the pre- and postsession.

Verbal WM with a Different Stimulus Set

Accuracy. In contrast to our expectations, multiple sessions with WM practice paired with active stimulation did not enhance verbal WM transfer performance more than sham stimulation (Group × Session, $F(1, 28) = 0.96$, $p = .34$ and Group × Session × Level n, $F(3, 84) = 0.61$, $p = .58$). Yet, a typical practice effect was observed with participants performing significantly better on the transfer letter n-back task in the postsession (Day 5 mean = 0.901, $SE = 0.013$) compared with the presession (Day 0 mean = 0.804, $SE = 0.017$; main effect Session, $F(1, 28) = 35.39$, $p < .001$) and at lower compared with higher levels of n (main effect Level n, $F(3, 84) = 71.91$, $p < .001$). Furthermore, a significant interaction between Session and Level n ($F(3, 84) = 9.41$, $p < .001$) likely reflects that the largest transfer gains were found for Levels 3 and 4 (see Figure 3). This is conceivable because participants practiced at these levels in the stimulation sessions.

RTs. Contrary to our prediction, analyses of the RT data also did not reveal enhanced performance on the transfer verbal WM tasks after active versus sham stimulation ($F(3, 84) = 0.17$, $p = .84$). All participants were faster in the postsession (Day 5 mean = 567 msec, SE = 24 msec) compared with the presession (Day 0 mean = 654 msec, SE = 21 msec; main effect Session, $F(1, 28) = 14.69$, $p = .001$), but this did not differ between the stimulation groups (Session × Group, $F(1, 28) = 0.35$, $p = .56$), thus indicating a general practice effect. Furthermore, a trend was observed toward faster RTs on the lower levels compared with the higher levels of n ($F(3, 84) = 2.69$, $p = .079$).

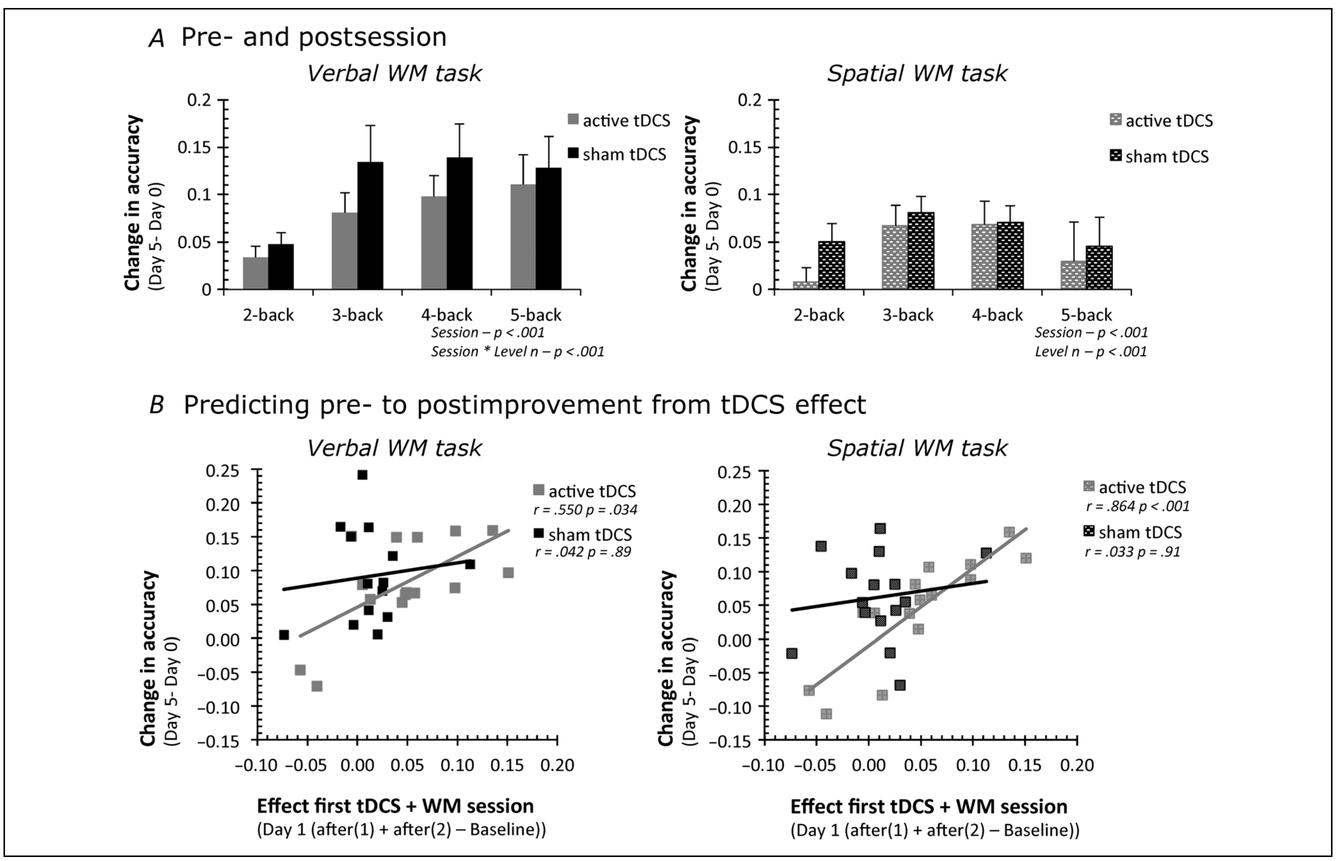

Figure 3. Transfer effects of active versus sham stimulation paired with WM training on the different WM tasks. (A) Both groups showed improvements on the verbal and spatial WM transfer task in the postsession compared with the presession, indicative of a general practice effect. However, at the group level, active tDCS did not result in greater improvements than sham stimulation. (B) Post hoc individual difference analyses revealed that, in the active stimulation group, participants that showed larger WM improvements in the first stimulation session also showed the largest pre- to postimprovements on both the verbal and spatial WM transfer task. Notably, this relationship was not observed in the sham stimulation group. Thus, tDCS may have enhanced WM functioning specifically in participants for which the tDCS was most effective. However, performance of two participants actually worsened after active stimulation in Session 1 and on both transfer tasks in the postsession. This raises the possibility that repeated stimulation paired with WM training can also impair WM functioning in some participants.

Spatial WM

Accuracy. A similar pattern was observed on the spatial WM transfer task. The amount of pre- to postimprovement in accuracy did not differ between the experimental groups (Group × Session, $F(1, 28) = 0.49$, $p = .49$; Group × Level n, $F(3, 84) = 1.01$, $p = .39$; Group × Session × Level n, $F(3, 84) = 0.43$, $p = .62$), indicating that anodal tDCS did not improve accuracy on the spatial WM task more than sham. Participants displayed significantly higher accuracy scores in the postsession (Day 5 mean = 0.855, SE = 0.015) compared with the presession (Day 0 mean = 0.803, SE = 0.014; main effect Session, $F(1, 28) = 15.66$, $p < .001$) and performed better at lower levels compared with higher levels of n (main effect Level n, $F(3, 84) = 119.88$, $p < .001$). The Session × Level n interaction was significant at trend level ($F(3, 84) = 2.88$, $p = .075$).

RTs. Again similar to the verbal WM transfer task, no differences in change in RT over time were found between the stimulation groups on the spatial WM transfer task (Group × Session, $F(1, 28) = 0.34$, $p = .57$; Group × Level n, $F(3, 84) = 0.70$, $p = .51$; Group × Session × Level n, $F(3, 84) = 0.49$, $p = .66$), indicating that anodal stimulation did not affect RT on the spatial WM transfer task differently from sham stimulation. Again, a practice effect was observed: participants were faster on the spatial WM task in the postsession (Day 5 mean = 535 msec, SE = 22 msec) compared with the presession (Day 0 mean = 594 msec, SE = 19 msec; main effect Session, $F(1, 28) = 9.18$, $p = .005$). At trend level, they were also faster for lower levels compared with higher levels of n (main effect Level n, $F(1, 28) = 2.60$, $p = .079$).

A Complex WM Task

Active stimulation was also not associated with greater improvements in performance on the complex WM transfer task. Total scores of the Ospan showed no difference between stimulation groups in the number of letters recalled between the pre- and postsession (Session × Stimulation Group, $F(1, 28) = 0.03$, $p = .86$) or between sessions (main effect Session, $F(1, 28) = 1.46$, $p = .24$). Also, no differences were observed in number of errors made in the mathematical operations on the postsession compared with the presession (main effect Session, $F(1, 28) = 0.26$, $p = .62$; Session × Group, $F(1, 28) = 0.48$, $p = .50$). However, despite our two versions of practice operations, post hoc analyses revealed that the time set to solve the math operations significantly differed between the pretest (Day 0 mean = 6771 msec, SE = 836) and the posttest (Day 5 mean = 5469 msec, SE = 483; main effect Session, $F(1, 28) = 4.92$, $p = .035$). However, this did not differ between the stimulation groups (Group × Session, $F(1, 28) = 0.00$, $p = .99$) and is thus not likely to affect our observed lack of tDCS effects on Ospan task performance.

In summary, our analyses of the transfer task data showed that participants were more accurate and faster in the postsession compared with the presession on both the verbal and spatial WM transfer tasks, but their performance did not increase on the complex WM span task. Moreover, the extent of improvement did not differ between the experimental groups. Thus, contrary to our prediction, combined anodal tDCS and verbal WM practice was not associated with larger general WM benefits at the group level, as measured by our transfer tasks.

Individual Difference in tDCS Respondence and Transfer

Recent research shows that the effects of tDCS can vary greatly across individuals (London & Slagter, 2015; Berryhill & Jones, 2012). We therefore explored if the extent to which a participant benefitted from combined stimulation and WM practice (i.e., tDCS respondence) could predict pre- to postenhancement on the transfer tasks. As no changes were found on the Ospan, analyses were done for the verbal and spatial WM transfer tasks only. As the effects of tDCS on behavior were only found in the two blocks after stimulation in the first session, tDCS respondence was computed as the difference in accuracy between the before (baseline) block and the average of both blocks after stimulation. Pre- to post-session improvements were calculated per transfer task as the difference in accuracy, collapsed over level of n. One participant (from the sham group) showed exceptionally large improvements pre to post on the letter WM task (>4 STD from the mean) and was therefore excluded from the analyses.

Interestingly, for the verbal WM task pre- to postimprovement significantly correlated with tDCS respondence in the active stimulation group (Spearman's rho = .550, $p = .034$) but not the sham stimulation group (Spearman's rho = −.042, $p = .89$). A Fisher transformation showed a trend level difference between these correlations (z = 1.58, $p = .05$ [one-tailed] calculated with vassarstats.net), indicating that only in the active group participants with the largest verbal WM improvements in the stimulation session also showed the largest pre- to postincreases on the verbal WM transfer task.

What's more, for the spatial WM transfer task tDCS respondence in the first session also predicted pre- to postimprovements in the active stimulation group (Spearman's rho = .864, $p < .001$), but not the sham stimulation group (Spearman's rho = .033, $p = .91$). Again Fisher's transformation showed a significant difference between these correlation coefficients (z = 3.06, $p = .001$), showing that only in the anodal group, participants with the largest verbal WM enhancements showed the largest improvements on the spatial WM transfer task (see Figure 3B).

Notably, closer inspection of the data showed that two participants in the active stimulation group showed WM decrements both in the first stimulation session and between the pre- and posttest sessions. This may indicate that active stimulation may have actually impaired WM function in some individuals. To determine to what extent these participants contributed to the observed correlation between change in performance during active stimulation and change in performance on the transfer tasks, we ran a control analysis in the active group in which we excluded these participants. For the verbal WM transfer task, this resulted in a nonsignificant correlation (Spearman's rho = .313, p = .297). However, for the spatial WM transfer task, the correlation remained highly significant (Spearman's rho = .819, p = .001) and was furthermore still significantly different from the correlation observed for the sham stimulation group (z = 2.57, p = .0052).

Care should be taken in interpreting these data because of the small sample size of the current study, but at the very least these results stress the importance of looking at individual differences, as tDCS may improve WM in some individuals but impair WM in others.

DISCUSSION

The current study aimed to investigate the effects of multiday tDCS stimulation over lDLPFC on verbal WM performance. More specifically, we examined if three sessions with anodal stimulation (1 mA for 20 min) over lDLPFC (anode F3, cathode rOFC) combined with a challenging verbal WM task may result in cumulative as well as general WM improvements. There were five main findings. First, stimulation improved verbal WM in the first stimulation session, replicating findings from previous single-session studies (Bennabi et al., 2014). Notably, these effects were only apparent after but not during stimulation. Furthermore, the greater WM enhancements observed in the first stimulation session in the stimulation (but not sham) group were still present 24 hr later, indicating that the effects of tDCS on WM may not simply reflect short-lived changes in neuronal excitability. However, third, no additional enhancements in verbal WM performance were observed in the subsequent two stimulation sessions. This is in contrast to our expectations but corroborates previous reports with different stimulation setups in which also no additional effects of tDCS were observed in multiple daily stimulation sessions (Richmond et al., 2014; Lally et al., 2013). Fourth, participants improved on both the verbal and spatial WM transfer tasks, but not the complex WM span, but not significantly more so after they had received anodal stimulation compared with sham. Thus, in line with previous findings (Richmond et al., 2014; Martin et al., 2013), we found no evidence that anodal stimulation might lead to enhanced transfer of WM benefits at the group level. However, fifth and finally, individual difference analyses revealed that within the group that had received anodal stimulation, gains in verbal WM in the first stimulation session predicted pre- to posttraining improvements on both the verbal and spatial WM transfer tasks. This relationship was not found for participants that received sham stimulation. Although this cross-subject relationship should be interpreted with caution because of our small sample size, it may provide support for the idea that when effective, anodal tDCS over lDLPFC paired with WM practice may induce WM improvements that outlast the temporary effects of stimulation as well as transfer to a different modality and thus may reflect true changes in WM functioning. Yet, two participants in the active stimulation group actually displayed worse WM performance after stimulation and on the verbal and spatial WM transfer tasks. This observation highlights the importance of taking individual differences into account and the need for future studies to determine the factors that may underlie such individual differences in tDCS respondence. These studies should also examine the extent of potential negative effects of tDCS in some individuals.

Contrary to our expectations, on the second and third day of stimulation, anodal tDCS did not further boost verbal WM in our participants. Hence, also with an electrode setup identical to the one that has repeatedly shown effective verbal WM improvements in single-session studies (with the reference over rOFC) and more optimal parameters in the form of current strength (1 mA), duration (20 min), and task paired with stimulation (verbal WM on a challenging level), anodal tDCS only significantly enhanced WM compared with sham on the first of three consecutive days of stimulation. Several reasons may account for this.

First, in previous research with multisession tDCS and WM, it has been proposed that tDCS may be only effective in boosting the "early" phases of learning (Richmond et al., 2014; Lally et al., 2013). Indeed, for example in the different domain of threat detection learning, Bullard et al. (2011) found that participants who received tDCS during the first of 2 hr of training showed greater improvements than those that received stimulation during the second hour. Importantly, in the current study, participants had already performed the task for over 60 min (in the presession and the first baseline block of the task) before stimulation was admitted. Therefore, they supposedly were already beyond these very first stages of learning in the first stimulation session, making it unlikely that this explanation accounts for the current results.

Second, it has been speculated that, rather than improving actual WM functioning, tDCS-induced enhancements may be the result of strategy learning, which presumably takes effect rapidly but also reaches ceiling level quickly. Among other functions, the lDLPFC has been related to strategic processes (Bor, Duncan, Wiseman, & Owen, 2003; MacDonald, Cohen, Stenger, & Carter, 2000). Yet, if anodal tDCS would facilitate strategy learning only, one would not expect such benefits to transfer to tasks that rely on different strategies. Our participants consistently reported the use of a verbal strategy in the verbal WM task and nonverbal strategies in the spatial WM task. Still, we found that in those participants that benefited most from the stimulation paired with practice, improvements in verbal WM transferred to a spatial version of the task. This makes it unlikely that the here observed effects of tDCS reflect verbal WM strategy learning solely.

Third and finally, the absence of tDCS effects in the second and third day of stimulation may be related to the time implemented between stimulation sessions. tDCS effects on behavior that are caused by neuroexcitability changes are generally assumed to have worn out after minutes (with very short stimulation durations) or a few hours (>10 min of stimulation; Nitsche et al., 2008; Nitsche & Paulus, 2000). Spacing the sessions with 24 hr in between has therefore generally been considered safe in ensuring that neuroexcitability effects from previous sessions have worn out before new stimulation is admitted. Interestingly, in the current study WM performance was significantly higher in the baseline block in the second stimulation session in the anodal compared with the sham group. This may reflect that anodal tDCS induced longer lasting effects or it could indicate that neuroexcitability levels in the stimulated areas had in fact not yet returned to baseline. Interestingly, a new study with seven sessions of anodal tDCS over DLPFC paired with a spatial WM training found significantly larger WM gains between the third and fourth session in participants in which these sessions were separated by a weekend (i.e., 72 hr) than for those that received them on consecutive days (i.e., 24 hr; Au et al., 2016). This implies that, to achieve cumulative tDCS effects, multiple stimulation sessions may in fact need to be spaced more than 24 hr apart.

However, as the study by Au and colleagues (2016) included no daily baseline measure, it remains unclear whether their results should be interpreted as a larger within-session effect of tDCS or stems from better learning consolidation after 72 hr of "rest" time. Similar to muscles after physical exercise, it is conceivable that for optimal learning to take place in brain regions a minimum of rest time is required to consolidate effects. Unfortunately, little is currently known about optimal intervals for enhancing cognitive functioning with training or tDCS (Goldsworthy, Pitcher, & Ridding, 2015). Furthermore, although the current study did not find behavioral effects of tDCS in subsequent sessions, we do not know whether the same learning effects would have been found with only one of the three daily stimulation sessions. Research that systematically investigates the effects of spacing of stimulation sessions on WM performance is necessary to determine which multiple session protocol(s) combining tDCS and WM training may be most effective to cumulatively and lastingly enhance WM functioning. In addition to such stimulation parameters, these studies should also take other variables into account that may facilitate transfer of WM learning, such as stimulus and task variability and optimal levels of arousal (e.g., see Slagter, 2012).

In line with previous studies (Richmond et al., 2014; Martin et al., 2013), at the group level anodal tDCS over lDLPFC was not associated with greater pre- to post-WM improvements than sham stimulation. This may seem contrary to the conclusion of a recent meta-analysis study by Mancuso et al. (2016) that reported a small but significant effect of left DLPFC anodal stimulation coupled with WM training. However, this analysis, based on 10 studies in total, included six single-session studies. It is hence possible that the reported effect is completely driven by effects of anodal stimulation in the first session. This would be quite in line with our finding of an effect of anodal tDCS on WM performance in the first session only.

However, additional individual differences analyses showed that the degree to which tDCS combined with WM practice was effective in boosting WM in the first session, predicted gains on the verbal and spatial WM transfer task posttraining. Our results thereby add to the growing number of studies that report that the effects of tDCS may vary substantially across individuals (e.g., London & Slagter, 2015). Notably, two participants in the active stimulation group actually displayed decrements in WM performance, suggesting that anodal lDLPFC stimulation may also impair WM performance in some individuals. Without these participants, the relationship between individual tDCS respondence and individual change in performance on the spatial WM transfer task remained significant, but this was no longer the case for the verbal WM transfer task. The latter could reflect reduced statistical power but may also indicate that this relationship was spurious. Future studies with larger samples sizes are necessary to determine whether tDCS respondence during WM training determines the strength of transfer effects. This research should also include a stimulation-only (i.e., without WM training) group, so that effects of tDCS and WM training can be better separated. Lastly, future studies should determine why some individuals may and why others may not respond to stimulation or even in a negative manner.

Several explanations have been proposed for individual differences in tDCS respondence. First, a recent modeling study has indicated that current flow may be strongly affected by individual differences in anatomy, skull thickness, and folding of the cortex (Opitz et al., 2015). As a result of this, standard tDCS setups may be more or less effective in affecting cognitive functioning, simply because they are more or less successful in delivering current to the target brain area. Interestingly, a recent study indeed observed a direct relationship between individually simulated current density values in the DLPFC and behavioral effects of prefrontal anodal tDCS on verbal WM (Kim et al., 2014). Second, the effect of tDCS may be dependent on the "baseline" activation level of an area, needing some activity to "grasp" on (Berryhill & Jones, 2012), but also interacting with a delicate optimal balance of activity levels for the best cognitive performance (London & Slagter, 2015). Third, and likely related to this, it has been suggested that baseline excitability/inhibitory balances in the stimulated cortex (reflected in GABA/glutamate concentration ratios) may predict the effectiveness of tDCS (Krause, Márquez-Ruiz, & Cohen Kadosh, 2013). Future studies that combine stimulation with neuroimaging and current flow modeling are needed to shed more light on individual differences in tDCS respondence and help predict which participants may benefit most from tDCS and why, but also how possible negative effects of stimulation can be prevented.

As no neuroimaging was included, we can only speculate about the underlying mechanisms through which anodal tDCS over lDLPFC modulated WM functioning in our study. We expect the effects to stem primarily from changes in functioning in the left DLPFC itself, an area that is known to play a key role in WM. Furthermore, as activation in this region has been related both to verbal and spatial WM (Owen et al., 2005), it provides a logical neural basis for the transfer of stimulation effects we found to the spatial domain. Nonetheless, studies that combined tDCS with neuroimaging have reported more widespread changes after lDLPFC stimulation (Stagg et al., 2013; Keeser et al., 2011), making it likely that the tDCS effects on WM in the current study are not confined to lDLPFC alone but may also include other regions.

To conclude, repeated anodal tDCS over lDLPFC concurrent with a challenging verbal WM task improved verbal WM performance only in the first of three daily stimulation sessions. Furthermore, individual differences in respondence to stimulation paired with WM practice predicted the extent and direction of WM improvement on a verbal and spatial transfer task (2 days after stimulation). More research is needed to determine which individuals may benefit the most from stimulation and why some individuals may be negatively affected, as well as to determine the optimal spacing of sessions for WM learning to take place. With a growing aging population and WM that is known to decrease over the lifespan, future research in this direction may help delineate the optimal parameters to use tDCS most effectively to enhance WM functioning in a range of individuals.

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