Neuromodulation of cognitive-behavioral control

Jongkees, B.J.

Chapter Six

L-tyrosine administration modulates the effect of transcranial direct current stimulation on working memory in healthy humans

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Abstract

Transcranial direct current stimulation (tDCS) is an increasingly popular method of modulating cognitive functions in humans. However, some doubt its efficacy as findings are inconsistent or remain unreplicated. It is speculated dopamine (DA) might play an important role in this inconsistency, by determining the direction and strength of the cognitive-behavioural effects of tDCS. However, so far evidence for this hypothesis has been correlational in nature, precluding definitive conclusions. The present proof-of-principle study aimed at investigating a potentially causal role for DA in the effect of tDCS on cognition in healthy humans. In experiment 1 we aimed to replicate previous findings showing administration of DA's precursor L-Tyrosine (Tyr), presumably by inducing a modest increase in DA level, can enhance working memory performance as assessed with a verbal *N*-back task. In experiment 2 we investigated the effect of Tyr administration on bilateral tDCS over dorsolateral prefrontal cortex (DLPFC) and working memory. Experiment 1 showed Tyr administration enhances performance in a verbal *N*-back task. Experiment 2 showed Tyr modulates the effect of bilateral tDCS over DLPFC on working memory. Specifically, tDCS had opposite effects on performance depending on current direction through the brain and Tyr administration. The present study provides two major findings. First, we replicate Tyr's beneficial effect on verbal working memory. Second, our results indicate a causal role for DA in the effect of tDCS on cognition. For this reason, we encourage future studies to consider the modulating effect of DA, as a step towards more consistent and replicable results regarding the efficacy of tDCS.

Introduction

Transcranial direct current stimulation (tDCS) is an increasingly popular, non-invasive method for modulating cognitive functions in healthy individuals and psychiatric patients (Plewnia, Schroeder, & Wolkenstein, 2015). tDCS induces a polarity-dependent shift in the resting membrane potential of cortical neurons, altering their likelihood of firing (Nitsche & Paulus, 2000) and longer stimulation results in neuroplastic after-effects (Nitsche & Paulus, 2001; Nitsche, Nitsche, et al., 2003). However, some still doubt the efficacy of tDCS in enhancing cognitive-behavioral performance, as many findings have so far not been subject of replication, and the effects of tDCS have some variability (Horvath, Forte, & Carter, 2015a, 2015b), but see (Antal, Keeser, Priori, Padberg, & Nitsche, 2015). Although much variation in results could be due to methodological differences between studies (e.g. stimulation duration, electrode placement) (Plewnia et al., 2015), another source of variance might stem from dopamine (DA) function (Li, Uehara, & Hanakawa, 2015). This idea is corroborated by the finding that tDCS differentially affects individuals carrying certain DA-related genetic polymorphisms (Nieratschker, Kiefer, Giel, Krüger, & Plewnia, 2015; Plewnia et al., 2013). However, given the inherently correlational nature of genetic studies, it remains unconfirmed whether DA plays a causal role in modulating the cognitive-behavioral effects of tDCS. Previous studies investigating the effect of DA manipulations on tDCS focused only on the electrophysiological effects of tDCS in the motor cortex (Fresnoza, Paulus, Nitsche, & Kuo, 2014; Fresnoza, Stiksrud, et al., 2014; Kuo, Paulus, & Nitsche, 2008; Monte-Silva et al., 2009; Monte-Silva, Liebetanz, Grundey, Paulus, & Nitsche, 2010; Nitsche et al., 2006; Nitsche, Kuo, Grosch, et al., 2009). In contrast, no studies have investigated the effect of DA manipulations on the cognitive-behavioral effects of tDCS. Hence, it remains unclear whether DA can indeed contribute to variability of results obtained with prefrontal tDCS.

Although available evidence suggests DA modulates the physiological and behavioral effects of tDCS, it remains speculative why this is the case. One reason DA might interact with the acute effects of online tDCS (i.e. stimulation

coinciding with task performance) might be because both tDCS and DA target resting membrane potentials. Anodal (excitatory) and cathodal (inhibitory) stimulation increase and decrease resting membrane potentials respectively (Nitsche & Paulus, 2000), whereas DA enhances and reduces firing of neurons with high and low membrane potentials, respectively (Frank, 2005; Hernández-López et al., 1997). As such, DA is known to modulate task-induced cortical activity (Egan et al., 2001; Mattay et al., 2003) and, in turn, task-induced activity has been identified as a possible determinant of tDCS effects (Antal, Terney, Poreisz, & Paulus, 2007; Bortoletto, Pellicciari, Rodella, & Miniussi, 2015). This suggests changes in background and task-dependent neural firing might mediate an effect of DA on online tDCS. On the other hand, DA might modulate the after-effects of tDCS on cortical excitability by also acting on N-methyl-d-aspartate (NMDA) receptors. These receptors mediate neuroplasticity via long-term potentiation (LTP) and depression (LTD) (Lüscher & Malenka, 2012) and are thought to underlie the neuroplastic after-effects of tDCS (Liebetanz, Nitsche, Tergau, & Paulus, 2002; Nitsche et al., 2004; Nitsche, Fricke, et al., 2003). Previous animal studies show DA can facilitate the induction and consolidation of LTP and LTD, but results have varied for different receptor subtypes (Gurden, Takita, & Jay, 2000; Huang, Simpson, Kellendonk, & Kandel, 2004; Spencer & Murphy, 2000). In line with these findings, Nitsche et al. (2006) reported that pharmacologically blocking D2 receptors nearly abolished the after-effects of tDCS on cortical excitability, whereas activation of D2, and to a lesser-extent D1, prolonged these effects. Additionally, Kuo et al. (2008) demonstrated L-dopa administration turned an excitability enhancement due to anodal tDCS into a diminishment, whereas the inhibitory after-effects of cathodal tDCS were prolonged. Taken together these findings establish a role for DA in the acute and long-term physiological effects of tDCS, but so far studies investigating if and how this translates to cognition and behavior remain scarce.

Hence, in the present study we set out to clarify the (potentially causal) relation between DA and the cognitive-behavioral effects of prefrontal tDCS.

To this end we investigated whether a modest increase in DA in healthy individuals modulates the effect of tDCS on (verbal) working memory (WM), a core cognitive function (Miyake et al., 2000) often investigated in relation to tDCS. Slight increases in DA level can be achieved by administration of DA's biochemical precursor l-tyrosine (Tyr), which can enhance a variety of DA-related cognitive functions in humans (Jongkees, Hommel, Kühn, & Colzato, 2015). Thus we reasoned if a simple DA manipulation such as Tyr administration would modulate the effect of tDCS on WM, then this would provide first tentative support for a causal role of DA in the cognitive-behavioral effects of tDCS. Following earlier studies on a DA manipulation on tDCS (Kuo et al., 2008; Nitsche et al., 2006), we have restricted our investigation to the after-effects of tDCS.

We applied bilateral tDCS over the dorsolateral prefrontal cortex (DLPFC), a region strongly implicated in WM (Curtis & D'Esposito, 2003) and often targeted with tDCS. Many studies show WM improvements with tDCS by applying anodal stimulation over the left DLPFC, with the cathodal return electrode being placed over the contralateral orbital region (Fregni et al., 2005; Hoy et al., 2013; Jeon & Han, 2012; Mulquiney, Hoy, Daskalakis, & Fitzgerald, 2011; Ohn et al., 2008; Oliveira et al., 2013; Teo, Hoy, Daskalakis, & Fitzgerald, 2011) or symmetrically over the right DLPFC (Oliveira et al., 2013). Stimulation with the opposite montage, i.e. with the cathode over left DLPFC, is less-investigated but may impair performance (Marshall, Mölle, Siebner, & Born, 2005), although this result was obtained with intermittent (15s on/off) rather than the more common continuous stimulation. Given this pattern of results we hypothesized that, after placebo intake, individuals receiving anodal over left, cathodal over right (AL-CR) stimulation would show higher WM performance than those receiving cathodal over left, anodal over right (CL-AR) stimulation. Given the hypothesized causal role of DA in the effects of tDCS, we expected a modulation of this pattern of results after administration of Tyr.

In short, we aimed to provide first tentative evidence supporting a causal role for DA in the cognitive-behavioral effects of tDCS by investigating

whether administration of Tyr modulates the effects of two tDCS montages on WM as assessed in a verbal *N*-back task.

Methods

Overview

We performed two separate experiments. To support the notion Tyr modestly enhances WM performance, in experiment 1 we aimed to replicate previous findings showing beneficial effects of Tyr administration on WM performance (Colzato, Jongkees, et al., 2013) in a double-blind between-subjects design. Participants consumed either 2.0 g of Tyr or placebo and 1 h later, when plasma Tyr levels start to peak (Glaeser et al., 1979), their WM performance was tested on a verbal *N*-back task. In experiment 2 we assessed the interaction between two tDCS montages (AL-CR vs. CL-AR) and administration of Tyr or placebo in a single-blind, between-subjects design. As in experiment 1, participants consumed either 2.0 g of Tyr or placebo. 1 h later they received 15 min of either AL-CR or CL-AR stimulation over bilateral DLPFC and subsequently their WM performance was tested using the same *N*-back task as in experiment 1. The studies conformed to the ethical standards of the declaration of Helsinki, the protocols were approved by the local ethical committee (Leiden University, Institute for Psychological Research), and volunteers signed an informed consent form before participation.

Experiment 1

Participants

36 students were recruited from Leiden University and randomly assigned to one of two groups: placebo or Tyr. Each group consisted of 18 participants. The two groups were comparable with respect to age, $F(1, 34) = 3.42$, $p = .073$, weight, $F(1, 34) = .44$, $p = .513$, body-mass index (BMI), $F(1, 34) = .287$, $p = .595$, and identical with respect to gender distribution. Group demographics are shown in Table 1. Participants were screened individually using the Mini International Neuropsychiatric Interview (M.I.N.I.) (Sheehan et al., 1998). The M.I.N.I. is a well-established brief diagnostic tool in clinical, drug, and stress

research that screens for several psychiatric disorders and drug use (Colzato & Hommel, 2008; Colzato, Kool, & Hommel, 2008; Sheehan et al., 1998). As such, all participants were screened for physical and mental health problems. Individuals with recent or regular drug use were excluded from participation. One exception to this was hormonal contraceptive medication, which all female participants had to be using regularly to limit fluctuations in hormone levels associated with the menstruation cycle as these can influence DA function and thereby confound results related to DA (Colzato & Hommel, 2014; Czoty et al., 2009; E. Jacobs & Esposito, 2011). The specific type or brand of contraception was not recorded. None of the participants reported any health problems.

Table 1. Group demographics for Experiment 1 and 2.

	Experiment 1				Experiment 2							
					AL-CR				CL-AR			
	Placebo		Tyrosine		Placebo		Tyrosine		Placebo		Tyrosine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age (years)	20.8	1.9	22.2	2.4	20.8	2.0	19.7	1.7	20.7	2.3	20.9	1.4
Weight (kg)	62.7	7.6	64.1	4.7	63.2	6.1	62.5	8.1	65.8	9.3	68.5	9.7
BMI (kg/length2)	21.6	2.3	22.0	2.1	22.3	2.5	21.5	2.1	22.6	2.3	22.7	2.4
Female/male ratio	17/1		17/1		15/3		16/2		15/3		15/3	

Task design

The *N*-back task is conceptualized to assess WM performance (Kane, Conway, Miura, & Colflesh, 2007) and, indeed, is often used in tDCS studies investigating WM (Au et al., 2016; Fregni et al., 2005; Hoy et al., 2013; Mylius et al., 2012; Ohn et al., 2008; Oliveira et al., 2013; Teo et al., 2011; Zaehle, Sandmann, Thorne, Jäncke, & Herrmann, 2011). The present study used a letter-based, i.e. verbal version of the task (Colzato, Jongkees, et al., 2013). A 1-back condition might be too easy to find positive effects of Tyr (Colzato, Jongkees, et al., 2013) and previous studies on tDCS and the *N*-back task have primarily used 2-back and 3-back conditions. Hence to keep the experiments comparable we included both 2-back and 3-back conditions in experiment 1 and 2. The 2-back condition was always presented first.

Stimuli were presented in the middle of a computer screen with a refresh rate of 60 Hz and a 800 x 600 resolution using E-Prime 2.0 software. Participants were comfortably seated approximately 50 cm from the screen while wearing headphones. Responses were given using the 'z' and 'm' buttons of a QWERTY keyboard. Mapping of response buttons to target (i.e. repetition) and non-target (i.e. non-repetition) was counterbalanced across participants in each group. After an incorrect or belated response (latency longer than 1000 ms) a brief tone was presented to signal a mistake. Both the 2-back and the 3-back conditions consisted of two blocks of 51 + n trials. For example, a 2-back block consisted of 53 trials. Regardless of the current load condition, each block comprised 21 targets and 30 non-targets. All participants performed the 2-back condition first and then the 3-back condition, and each n-back condition was preceded by 17+n practice trials (7 targets and 10 targets).

Aside from parameters such as hit rates and correct rejections, we were mainly interested in target sensitivity, indexed by d' prime derived from signal detection theory (Swets, Tanner, & Birdsall, 1961). This informative measure combines hit and false alarm rates and thus provides an index of the ability to discriminate targets from non-targets, with higher scores signaling selective, correct reporting of targets, and thus better WM performance. Hence we

expected higher d' prime scores after Tyr administration. d' prime was calculated, and perfect scores were corrected for, as described earlier (Colzato, Jongkees, et al., 2013). One participant had a perfect hit rate and another had zero false alarms.

Tyrosine administration

To induce a moderate increase in DA we administered DA's precursor Tyr. Upon consumption Tyr is converted into L-dopa, which is subsequently converted into DA. Consistent with the hypothesis that Tyr administration increases DA, animal studies showed increased levels of prefrontal DA and homovanillic acid (HVA), the main metabolite of DA, after Tyr intake (Tam et al., 1990; Tam & Roth, 1997). Although in vivo, direct assessment of DA in humans is difficult, Tyr administration has been shown to significantly elevate levels of HVA in the spinal fluid of Parkinson's patients, suggesting an increase in DA (Growdon et al., 1982).

Previous studies showed doses of Tyr as low as 2.0 g have positive effects on WM performance as measured using the *N*-back task (Colzato, Jongkees, et al., 2013) and a variety of other cognitive functions (for a review, see Jongkees et al., 2015), suggesting this dose is sufficient for inducing a modest but functionally relevant increase in DA level. With the aim of replicating these findings, we administered 2.0 g of Tyr in the present study. The neutral substance microcrystalline cellulose was used as placebo (Thomas et al., 1999). Tyr or placebo was dissolved in 400 mL of orange juice.

As we did not adjust the dosage of Tyr to the individual participant's weight and BMI, this might have led to variation in response to the administration due to different substance concentration levels. To control for this source of variance we included BMI as covariate in our analyses.

Procedure

Participants came to the lab in the morning, having fasted since 10 o'clock in the evening prior to participation (Cuche et al., 1985; Glaeser et al., 1979). Informed consent was obtained and BMI was measured. Subsequently

participants consumed 2.0 g of Tyr or placebo dissolved in 400 mL orange juice. Afterwards they were offered apples and oranges, which contain negligible amounts of Tyr, to prevent strong hunger. 1 h after finishing the juice participants started the *N*-back task, which took approximately 20 min. Lastly, participants were debriefed and compensated for their participation with course credit or €10.

Statistical analysis

To assess the effect of Tyr intake on WM performance we conducted repeated measures ANCOVA with administration (placebo vs. Tyr) as between-subjects factor, WM load (2-back vs. 3-back condition) as within-subjects factor, BMI as covariate, and d' prime as the dependent measure. Similar analyses were performed using hit, false alarm, correct rejection, and miss rates, and reaction times (RT) as dependent measures.

Experiment 2

Participants

72 right-handed students were recruited from Leiden University. Participants were randomly assigned to one of four groups: AL-CR stimulation plus placebo, AL-CR stimulation plus Tyr, CL-AR stimulation plus placebo, or CL-AR stimulation plus Tyr. Each group consisted of 18 participants, which is comparable with—if not more than—previous tDCS studies on WM (Fregni et al., 2005; Hoy et al., 2013; Jeon & Han, 2012; Marshall et al., 2005; Mulquiney et al., 2011; Mylius et al., 2012; Ohn et al., 2008; Teo et al., 2011) and previous studies investigating the effect of a DA manipulation on the electrophysiological effects of tDCS (Fresnoza, Paulus, et al., 2014; Fresnoza, Stiksrud, et al., 2014; Kuo et al., 2008; Monte-Silva et al., 2009, 2010; Nitsche et al., 2006; Nitsche, Kuo, Grosch, et al., 2009). The four groups were comparable with respect to age, $F(3, 68) = 1.59$, $p = .201$, weight, $F(3, 68) = 1.89$, $p = .140$, BMI, $F(3, 68) = 1.00$, $p = .400$, and gender distribution, $X^2(3) = .32$, $p = .956$. Group demographics are shown in Table 1. Participants were again selected using the M.I.N.I (Sheehan et al., 1998) and underwent the same

health screening as described in experiment 1. Additionally, individuals with implants such as pacemakers, any kind of metal in their body, or skin conditions were excluded for safety reasons concerning the tDCS. None of the participants reported any health problems.

Task design

The same letter-based, verbal *N*-back task used in experiment 1 was used to assess WM performance. In line with our hypotheses in the Introduction, after placebo intake, we expected higher d' prime scores when stimulating with a typically performance-enhancing AL-CR, as compared to a typically impairing CL-AR montage. Given the hypothesized role of DA in the effects of tDCS, we expected these results to be modulated by Tyr.

One participant in the CL-AR plus Tyr group achieved a perfect hit rate in the 2-back condition and hence this score was corrected (Colzato, Jongkees, et al., 2013). No participant achieved zero false alarm rates.

Transcranial direct current stimulation

Two electrodes of 35 cm^2 (5 cm x 7 cm) were placed over DLPFC in a bilateral bipolar-balanced montage (Nasseri, Nitsche, & Ekhtiari, 2015), i.e. in symmetrical positions. Although many tDCS studies on WM have previously placed the return electrode over the contralateral supraorbital region, we opted for symmetrical positioning of electrodes to avoid uncertainty over where in the supraorbital region the electrode was placed. Additionally, and more importantly, this positioning served to maximize the likelihood current direction through the brain would be comparable across participants, thus reducing potential variability in response to the stimulation. For each individual participant the DLPFC was located using the international 10/20 system for placing electrodes on the scalp (Jasper, 1958). Accordingly, for the AL-CR montage the anode and cathode were placed over F3 and F4, respectively. For the CL-AR montage this placement was reversed.

Stimulation consisted of a current of 1000 μA delivered by a DC Brain Stimulator Plus (NeuroConn, Ilmenau, Germany), a device complying with the

Medical Device Directive of the European Union (CE-certified). The current was built up during a fade-in of 10 s, after which stimulation lasted for precisely 15 min and then ended with a 10 s fade-out. Impedance was below 15 kΩ throughout the stimulation. The after-effects of 15 min of tDCS typically last 1 h (Nitsche et al., 2008). We assessed WM performance off-line, that is after stimulation had finished. This mirrors the design of previous studies investigating the effect of DA manipulations on tDCS-induced cortical excitability. For example, Nitsche et al (2006) and Kuo et al. (2008) administered DA agents to participants, then stimulated the motor cortex and afterwards measured cortical excitability. Additionally, it is important to consider the effects of online tDCS may be particularly state-dependent, i.e. there may be variation in response due to differences in task-induced activity or baseline performance across participants (Antal et al., 2007; Bortoletto et al., 2015). To avoid minimize such a confound in our results we opted for offline assessment of WM performance.

The experience of side-effects due to tDCS was assessed through self-report ratings for the following symptoms: head ache, neck pain, nausea, muscle contractions in the face or neck, stinging sensation under the electrodes, burning sensation under the electrodes, and a nonspecific, uncomfortable feeling. Consistent with previous studies the most prominent side-effects were stinging and burning sensations under the electrodes (Bikson, Datta, & Elwassif, 2009), although none of the participants voiced major complaints.

Tyrosine administration

As in experiment 1, we administered 2.0 g of Tyr or a placebo dissolved in 400 mL orange juice.

Procedure

The experimental procedure was similar to experiment 1. Participants came to the lab in the morning, having fasted since 10 o'clock in the evening prior to participation (Cuche et al., 1985; Glaeser et al., 1979). Informed consent was obtained and BMI was measured. Subsequently participants consumed 2.0 g

of Tyr or placebo dissolved in 400 mL orange juice. Afterwards they were offered apples and oranges to prevent strong hunger. 45 min after finishing the juice the tDCS montage was applied. After mounting the electrodes on the head, which took approximately 10 to 15 min, at precisely 1 h after Tyr or placebo administration the stimulation was started. Once the 15 min stimulation had finished the montage was removed. The participants then started the *N*-back task, which took approximately 20 min. Afterwards, participants' experience of any side-effects due to tDCS was rated. Lastly, participants were debriefed and compensated for their participation with course credit or €15.

Statistical analysis

To assess the effect of tDCS combined with Tyr we performed repeated measures ANCOVA with montage (AL-CR vs. CL-AR) and administration (placebo vs. Tyr), as between-subjects factors, WM load (2-back vs. 3-back condition) as within-subjects factor, d' prime as the dependent measure, and BMI as covariate. Similar analyses were performed for hit, false alarm, correct rejection, and miss rates, and RT as dependent measures.

A significant interaction between montage and administration was further investigated with additional ANCOVAs to disentangle this effect on WM performance according to our main hypothesis. Control comparisons between experiment 1 and 2 were performed to further clarify the effect of combined tDCS and Tyr relative to administration of only Tyr.

Results experiment 1

Target sensitivity

To replicate the positive effect of Tyr intake on WM performance, participants completed a letter-based *N*-back task after they consumed either placebo or Tyr. For d' prime scores, there was a significant effect of WM load, $F(1, 33) = 10.45$, $p = .003$, partial $\eta^2 = .241$. This indicates significantly higher d' prime scores in the 2-back condition ($M = 2.74$) than in the 3-back condition ($M = 1.99$). More importantly, there was a main effect of administration, $F(1, 33) =$

6.94, p = .013, partial η^2 = .174. Target sensitivity was significantly higher after intake of Tyr (M = 2.69) than after placebo (M = 2.05). There was no significant interaction between WM load and administration, $F(1, 33) = 1.01$, p = .321, suggesting the effect of Tyr was comparable in the 2-back and 3-back conditions.

Other N-back parameters

Results for hits, misses, correct rejections, false alarms, and RT were similar to d' prime. Means are listed in Table 2.

For hits and misses there was again a main effect of administration, $F(1, 33) = 5.30$, p = .028, partial η^2 = .138. This suggests significantly higher hit rates and less misses after intake of Tyr (M = .86 and .14, respectively) than after placebo (M = .78 and .22, respectively). Similarly, for correct rejections and false alarms there was also a main effect of administration, $F(1, 33) = 6.81$, p = .014, partial η^2 = .171. This indicates significantly higher correct rejection and lower false alarm rates after Tyr intake (M = .92 and .08, respectively) than after placebo (M = .84 and .16, respectively).

For RT on target trials, there was no significant effect of administration, $F(1, 33) = 3.00$, p = .093, partial η^2 = .083. However, for RT on non-target trials there was a significant effect of administration, $F(1, 33) = 4.97$, p = .033, partial η^2 = .131, indicating faster responses on non-target trials after Tyr intake (M = 559) than after intake of placebo (M = 609).

BMI

For the sake of clarity we discuss the BMI results in this separate section. There were no significant main effects of BMI, all $p > .05$. Interestingly, there was a significant interaction between BMI and load when analyzing the d' prime scores, p = .015. However separate regression analyses for the two load conditions (2-back and 3-back) with BMI as predictor and d' prime as dependent measure revealed no significant effect of BMI on d' prime in either condition, ps > .05. Plotting the data revealed the interaction was likely driven by a tendency for d' prime scores to be lower in the 2-back condition with

increasing BMI, whereas this tendency was not observed in the 3-back condition. Lastly, in separate analyses we confirmed the effect of BMI did not differ in our two groups as indicated by a nonsignificant BMI and group interaction, *p*s > .05.

Table 2. Parameters of the N-back task in Experiment 1

	Placebo		Tyrosine	
	Mean	SD	Mean	SD
Hits*				
2-back	.83	.15	.91	.07
3-back	.72	.15	.82	.12
Misses*				
2-back	.17	.15	.09	.07
3-back	.28	.15	.18	.12
Correct rejections*				
2-back	.88	.12	.93	.05
3-back	.81	.11	.90	.06
False alarms*				
2-back	.12	.12	.07	.05
3-back	.19	.11	.10	.06
Reaction times 2-back				
Target	541	83	506	71
Non-target*	568	90	528	65
Reaction times 3 back				
Target	606	86	544	68
Non-target*	608	78	579	60

* $p < .05$

Results experiment 2

Target sensitivity

To investigate whether Tyr modulates the effect of tDCS on WM, participants completed a letter-based *N*-back task after being administered either placebo or Tyr and having been stimulated for 15 min with an AL-CR or CL-AR montage. For d' prime, there was a significant effect of WM load, $F(1, 67) = 5.23$, $p = .025$, partial $\eta^2 = .072$. Specifically, scores in the 2-back condition ($M = 2.35$) were significantly higher than in the 3-back condition ($M = 1.67$). There were no significant interactions between WM load and montage, $F(1, 67) = .06$, $p = .811$, between WM load and Tyr administration, $F(1, 67) = .42$, $p = .520$, or between WM load and both montage and Tyr administration, $F(1, 67) = .01$, $p = .921$, suggesting the difference in difficulty between the 2-back and 3-back conditions was experienced similarly across the four groups.

More importantly, although we did not find a main effect of montage $F(1, 67) = 2.79$, $p = .099$, or Tyr administration, $F(1, 67) = .09$, $p = .771$, we did find a significant interaction between montage and Tyr administration, $F(1, 67) = 4.81$, $p = .032$, partial $\eta^2 = .067$. This indicates target sensitivity was modulated by the combination of tDCS and Tyr. The interaction is illustrated in Figure 1. To disentangle this interaction, we ran separate ANCOVAs for the placebo and Tyr groups. After placebo intake there was only a small, non-significant difference between the AL-CR and CL-AR montages in terms of d' prime ($M = 2.06$ vs. 1.99), $F(1, 33) = .10$, $p = .749$. However, after Tyr intake there was a larger, significant difference between the AL-CR and CL-AR montages in the opposite direction of typical results on tDCS and WM, $F(1, 33) = 6.50$, $p = .016$, partial $\eta^2 = .165$. That is, d' prime scores were higher after CL-AR stimulation was combined with Tyr ($M = 2.23$) than when the typically WM-enhancing AL-CR stimulation was combined with Tyr ($M = 1.75$), see Figure 1. This finding suggests inducing a moderately higher DA level in participants modulates the effect of tDCS on WM and, strikingly, leads a typically-enhancing stimulation montage to impair performance. This finding provides first evidence in favor of a causal role for DA in the cognitive-behavioral effects of prefrontal tDCS.

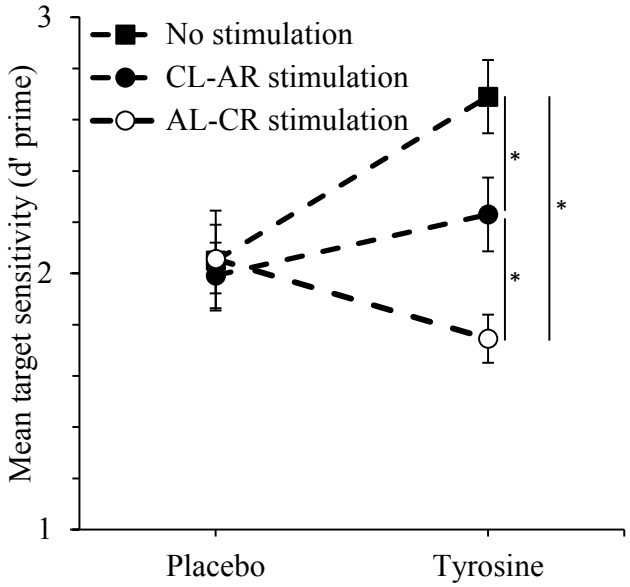

Figure 1. Mean d' prime scores in the *N*-back task for each group in experiment 1 (no stimulation plus placebo or Tyr) and experiment 2 (CL-AR stimulation plus placebo or Tyr and AL-CR stimulation plus placebo or Tyr). Scores are averaged across the two WM load conditions (2-back and 3-back). * $p < .05$

Other N-back parameters

Results for hits and misses showed similar patterns to d' prime. Means are listed in Table 3.

For hits and misses there was no main effect of montage, $F(1, 67) = 1.82$, $p = .182$, nor Tyr administration, $F(1, 67) > .001$, $p = .987$, but again there was a significant interaction between montage and Tyr administration, $F(1, 67) = 5.46$, $p = .022$, partial $\eta^2 = .075$. Additional ANCOVAs revealed a significant difference between the montages after Tyr intake, $F(1, 67) = 7.31$, $p = .011$, partial $\eta^2 = .181$. As with d' prime scores, the CL-AR group showed higher performance (i.e., more hits and less misses) than the AL-CR group when combined with Tyr, whereas no difference was observed when combined with placebo, $F(1, 33) = .40$, $p = .529$. There were no significant 2 or 3-way interactions between WM load, montage and Tyr administration, all ps > .398.

For correct rejections and false alarms there were no main effects of montage, $F(1, 67) = 2.25$, $p = .138$, or Tyr administration, $F(1, 67) = .41$, $p = .523$, nor an interaction between montage and Tyr administration, $F(1, 67) = 1.73$, $p = .193$. The only significant interaction involved WM load and montage, $F(1, 67) = 4.14$, $p = .046$, partial $\eta^2 = .058$, indicating responses to non-targets were modulated by tDCS montage and this modulation differed between the 2-back and 3-back conditions. A post hoc pairwise comparison of the two montages for each level of WM load revealed no significant difference between the two montages in the 2-back condition, $p = .909$, whereas the two montages did differ significantly in the 3-back condition, $p = .034$, partial $\eta^2 = .065$. Specifically, participants who were stimulated with an AL-CR montage showed overall less correct rejections and, correspondingly, more false alarms ($M = .81$ and .19, respectively) as compared to those stimulated with a CL-AR montage ($M = .85$ and .15, respectively), but only in the 3-back condition. It should be noted this interaction was independent of whether participants were given placebo or Tyr.

Table 3. Parameters of the N-back task in Experiment 2

	tDCS montage							
	AL-CR				CL-AR			
	Placebo		Tyrosine		Placebo		Tyrosine	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Hits*								
2-back	.84	.08	.80	.10	.83	.11	.87	.08
3-back	.75	.11	.71	.10	.71	.13	.76	.11
Misses*								
2-back	.16	.08	.20	.10	.17	.11	.13	.08
3-back	.25	.11	.29	.10	.29	.13	.24	.11
Correct rejections								
2-back	.89	.06	.89	.06	.88	.07	.89	.05
3-back^o	.83	.07	.79	.07	.85	.06	.85	.09
False alarms								
2-back	.11	.06	.11	.06	.12	.07	.11	.05
3-back^o	.17	.07	.21	.07	.15	.06	.15	.09
Reaction times 2-back								
Target	534	55	542	80	539	81	560	87
Non-target	602	61	578	62	581	69	595	71
Reaction times 3 back								
Target	555	67	566	64	562	67	567	84
Non-target	607	69	592	41	602	74	609	67

** $p < .05$ for difference AL-CR plus Tyr versus CL-AR plus Tyr*

^{o} $p < .05$ for difference AL-CR versus CL-AR, regardless of Tyr administration

For RT on target and non-target trials there were no significant effects of montage or Tyr administration, nor any 2 or 3-way interactions between WM load, montage, and Tyr administration, all *p*s > .327.

Control comparisons

We performed control comparisons to gain insight in how stimulation (AL-CR and CL-AR) combined with placebo or Tyr affected performance relative to administering placebo or Tyr without tDCS. To this end we performed two additional ANCOVA's in which we separately compared performance of the groups receiving placebo or Tyr in experiment 2 to the group that received placebo or Tyr in experiment 1, respectively. Since hits and misses followed the same pattern of results as d' prime, we only performed comparisons for the latter.

For the placebo groups, a repeated measures ANCOVA was performed with group (placebo-only vs. AL-CR stimulation plus placebo vs. CL-AR stimulation plus placebo) as between-subjects factor, WM load (2-back and 3-back condition) as within-subjects factor, BMI as covariate, and d' prime as dependent measure. There was no main effect of group, $F(1, 50) = .04$, $p = .960$, suggesting placebo plus tDCS did not affect performance as compared to administration of only placebo. Subsequently we performed the same analysis, but now with the Tyr-only group vs. AL-CR stimulation plus Tyr vs. CL-AR stimulation plus Tyr as between-subjects factor. This analysis did show a significant effect of group, $F(1, 50) = 11.84$, $p > .001$, partial $\eta^2 = .321$. All three groups differed significantly from each other, with participants in the Tyr-only condition having significantly higher d' prime scores than participants in the CL-AR stimulation plus Tyr condition, $p = .018$, and the AL-CR stimulation plus Tyr condition, $p < .001$, and with the CL-AR stimulation plus Tyr condition also showing higher scores than the AL-CR stimulation plus Tyr condition, $p = .022$.

BMI

For the sake of clarity we discuss the BMI results in this separate section. There were significant main effects of BMI only when comparing d' prime scores after CL-AR plus placebo versus CL-AR plus Tyr, p = .034, and when comparing d' prime scores for all three placebo conditions (AL-CR plus placebo, CL-AR plus placebo, and placebo-only), p = .032. In both analyses a higher BMI was associated with worse performance and we revisit this point in the discussion. Importantly, all other main effects of BMI were not significant nor did BMI interact significantly with load, all ps > .05. In separate analyses we confirmed BMI did not interact with tDCS montage and Tyr supplementation, indicating the effect of BMI was comparable in all groups, all ps > .05.

Discussion

The present study reports two major findings. First, we show Tyr administration, which presumably induces a modest increase in DA, enhances verbal WM performance as assessed in a letter-based *N*-back task. This finding replicates previous studies showing beneficial effects of Tyr on WM (for a review, see Jongkees et al., 2015). Second, we show that Tyr, and therewith presumably DA, modulates the effect of tDCS on verbal WM in a current direction-dependent manner. Whereas previous studies show AL-CR stimulation of DLPFC benefits WM performance (Fregni et al., 2005; Hoy et al., 2013; Jeon & Han, 2012; Mulquiney et al., 2011; Ohn et al., 2008; Oliveira et al., 2013; Teo et al., 2011) and CL-AR stimulation may impair performance (Marshall et al., 2005), in our study tDCS combined with Tyr led to a different pattern: CL-AR stimulation of the DLPFC led to higher WM performance than AL-CR stimulation when both are combined with Tyr. This finding is in line with previous genetic studies showing higher prefrontal DA is associated with differential responses to tDCS over DLPFC (Nieratschker et al., 2015; Plewnia et al., 2013) and also fits the finding that combining a DA agonist with anodal stimulation of the motor cortex inverts tDCS after-effects, leading to excitability diminishment rather than enhancement (Kuo et al., 2008).

These results provide first tentative evidence for a causal role of DA in modulating the cognitive-behavioral effect of tDCS over the prefrontal cortex. In doing so, this study supports the hypothesis that DA function may be one factor that contributes to variability in results of tDCS studies. This idea could have relevance for our null-finding in experiment 2: after placebo intake we found no difference in performance after AL-CR as compared to CL-AR stimulation, although the former typically enhances and the latter typically impairs WM. Many reasons may account for this null-finding. However, based on our finding that DA modulates the effects of tDCS, we speculate inter-individual variability in DA function might be one factor, either alone or in combination with others, that could explain this lack of difference. In order to prevent such difficult-to-interpret null-findings, we encourage future studies to take into account individual differences related to DA. Doing so would be an important step towards obtaining a clearer and consistent view of the efficacy of tDCS in modulating cognitive-behavioral performance.

As mentioned in the introduction, it remains unclear what the precise mechanism is that underlies the interaction between DA and tDCS. It might be DA, and by extension Tyr, affects excitability modulations by online tDCS via an influence on membrane potentials, as DA can enhance neural firing in neurons with high potentials while suppressing firing in neurons with low potentials (Frank, 2005; Hernández-López et al., 1997). Hence DA and Tyr may affect online tDCS, i.e. stimulation during the critical task, by modulating task-induced activity, which has been identified as a determinant of tDCS effects (Antal et al., 2007; Bortoletto et al., 2015). DA and Tyr may also affect neuroplasticity induced by tDCS, which may have occurred in the present study, by affecting the NMDA-receptors that are believed to underlie the after-effects of tDCS (Liebetanz et al., 2002; Nitsche et al., 2004; Nitsche, Fricke, et al., 2003). Indeed, D1 and D2 receptors have been shown to affect NMDA-receptor-mediated LTP and LTD, although results for D2 receptors have been inconsistent (Gurden et al., 2000; Huang et al., 2004; Spencer & Murphy, 2000).

Additionally, there are alternative but related hypotheses on the interaction between DA and tDCS and these are not necessarily mutually-exclusive. (i) Tanaka et al. (2013) showed cathodal tDCS increased DA in the rat striatum. As the relationship between DA and cognitive performance typically follows an inverted-U curve (Cools & D'Esposito, 2011), it has been argued tDCS might shift an individual's DA level towards an optimum associated with peak performance (Li et al., 2015). Thus, response to tDCS would depend in part on an individual's initial position relative to the optimal level of DA. This would be particularly relevant for the large body of tDCS studies in aging (Perceval, Flöel, & Meinzer, 2016), as the interaction between DA and tDCS might be magnified by the interplay between genes, DA function, and aging (Li, Lindenberger, & Bäckman, 2010; Lindenberger et al., 2008). (ii) One hypothesis, which might explain why cathodal tDCS of the left DLPFC in conjunction with Tyr led to higher WM scores than the same stimulation without Tyr, is the combination of high DA levels and cathodal stimulation may improve the signal-to-noise ratio in the brain (Kuo et al., 2008), allowing for more efficient neural function. Future studies may be able to validate these speculations, for example by showing enhanced cortical efficiency as indicated by reduced BOLD response during task engagement. (iii) An alternative mechanism relates to the calcium-dependence of tDCS-induced excitability alterations, DA activity, and task-related cortical activity alterations, probably transmitted via the glutamatergic system. Hereby it is important to acknowledge the effect of calcium enhancement on long-lasting cortical excitability alterations follows a non-linear rule. Low calcium enhancement results in reduced, whereas high calcium results in enhanced activity and excitability, but even larger calcium enhancement again reduces respective activity and excitability, possibly due to counter-regulative activation of potassium channels (Lisman, 2001; Misonou et al., 2004). Accordingly, it was shown recently that enhancing stimulation duration of anodal tDCS or combination of anodal tDCS with nicotine in non-smokers results in reduced cortical excitability, and that this process is calcium dependent (Lugon et al., 2015; Monte-Silva et al., 2013). Likewise, enhancing

stimulation intensity of cathodal tDCS switched the effects from excitability diminution to enhancement (Batsikadze, Moliadze, Paulus, Kuo, & Nitsche, 2013). One could speculate that in the present experiment task-dependent activation of neurons in combination with Tyr and anodal tDCS over left DLPFC led to a calcium overflow resulting in cortical activity reduction, and thus reduced performance, whereas the slight calcium increase probably caused by cathodal tDCS over left DLPFC in conjunction with Tyr optimized task-relevant calcium enhancement, and thus improved performance. Notably, performance after CL-AR stimulation combined with Tyr was still lower than after Tyr intake without tDCS, suggesting the effects of tDCS and a DA manipulation might not be additive and their combination might not be an enhancing method for all individuals. (iv) Lastly and particularly relevant for online assessment of performance, the effect of tDCS seems to strongly depend on task-induced activity, at least in the motor cortex (Antal et al., 2007; Bortoletto et al., 2015). Hence the same stimulation might have different effects depending on the extent to which neural activity is increased during task performance. On the other hand, DA is known to modulate task-induced activity and, indeed, the functional polymorphism in the COMT gene, which affects prefrontal DA degradation, is associated with different levels of prefrontal activation during WM engagement (Egan et al., 2001; Mattay et al., 2003). In line with this reasoning, the same COMT polymorphism modulates the effect of tDCS on executive function (Nieratschker et al., 2015; Plewnia et al., 2013). Hence we speculate individual differences related to genetically predetermined DA levels might influence the extent of neural activation during WM engagement and, in turn, this variability could lead to different effects of online tDCS.

Related to the topic of individual differences, we recommend future research to employ more adaptive WM tasks instead of 'static' tasks with the same conditions for each participant. For example, instead of having each participant perform a 2 and 3-back task, adaptive versions of the N-back task exist wherein *N* increases when participants perform well on the task and *N* decreases when they perform worse (e.g. Jaeggi, Buschkuehl, Shah, & Jonides,

2014). Such tasks can prove useful in future research that wishes to examine individual differences (and gain) in WM performance, as it can prevent ceiling effects from obscuring individual differences and practice effects from confounding effects of experimental manipulations in within-subjects designs. Also, it is interesting to note some of our analyses revealed a tendency for WM to be worse with increasing BMI, which is consistent with the idea obesity is related to impaired cognitive function (Prickett, Brennan, & Stolwyk, 2015). With respect to this finding it is important to note our experimental groups did not differ in terms of BMI. Future research might wish to further investigate the potential relationship between BMI and WM without having the results confounded by administration of Tyr or tDCS.

Future research may also wish to examine one notable difference between our and previous tDCS studies, which is that in the current experiment participants fasted overnight because of the Tyr administration. As of yet it is unclear if and how this might have affected the effects of tDCS and whether this fasting, perhaps in combination with Tyr and tDCS, may have contributed to the lack of an effect of tDCS on WM in the placebo conditions. Hence, it would be interesting for future studies to assess whether fasting can affect tDCS outcome.

Furthermore, it is important to acknowledge DA is probably one of many neurotransmitters relevant to tDCS effects, and thus future research should explore other neurotransmitter systems as well. As reviewed by (Stagg & Nitsche, 2011), a number of neurotransmitters are known to modulate the physiological effects of tDCS, but these investigations are restricted to the motor cortex and thus it remains unclear if and how these neurotransmitter determine cognitive-behavioral effects of tDCS. Important neurotransmitters that have been identified are glutamate and GABA, and their concentration levels change following anodal or cathodal stimulation over the motor cortex (Bachtiar, Near, Johansen-Berg, & Stagg, 2015; Soyoung Kim, Stephenson, Morris, & Jackson, 2014; Stagg et al., 2009). The consequent change in cortical excitation may facilitate or impair induction of LTP and LTD (Ziemann, Muellbacher, Hallett, & Cohen, 2001), and as such these neurotransmitters

may play a crucial role in the neuroplastic after-effects of tDCS (Stagg & Nitsche, 2011). Interestingly, individuals are known to differ in the balance between cortical glutamate and GABA, and this balance has been shown to predict response selection (de la Vega et al., 2014). Hence it would be interesting to see whether response to prefrontal tDCS can also be predicted based on individual differences in the glutamate/GABA balance. Other relevant neurotransmitters include acetylcholine, an increase of which abolished the after-effects anodal but prolonged the effects of cathodal tDCS (Kuo, Grosch, Fregni, Paulus, & Nitsche, 2007), and serotonin, an increase of which prolonged the after-effects of anodal tDCS but reversed the after-effects of cathodal tDCS from excitability diminishment into enhancement (Nitsche, Kuo, Karrasch, et al., 2009). Recently it was also shown noradrenaline modulates tDCS-induced plasticity, with different effects for acute and chronic pharmacological enhancement of noradrenaline activity (Kuo et al., 2016). This is particularly interesting when considering effects of Tyr administration may be mediated by DA and/or noradrenaline (Jongkees et al., 2015), as mentioned below. As such, future studies may wish to examine individual differences in terms of these neurotransmitters as well.

Before concluding, we wish to elaborate on some limitations of this study. First, in experiment 2 we did not include a sham-tDCS condition. Hence we cannot be sure the observed effects of tDCS are due to modulation of neural physiology or rather due to expectations of the participants evoked by the experience of mounting electrodes on the head and the accompanying tingling, burning sensations. However, we argue that an explanation of our results in terms of participants' expectations is unlikely as the placebo groups from both experiments were comparable in performance. If participants' expectations due to tDCS had indeed influenced our results, it would have likely resulted in differences between these groups.

The second limitation is we did not assess inter-individual variability in DA function, for example in terms of genetic polymorphisms. Although the present study aimed at finding proof-of-principle for the idea DA modulates prefrontal tDCS, not considering individual differences between our

participants may have led to the inability to replicate a beneficial effect of tDCS on WM. For this reason it would be valuable to replicate and validate our findings in future studies in which individual differences are taken into account.

The third limitation relates to the presumed effect of Tyr on catecholamine synthesis. Tyr is the precursor of both DA and noradrenaline (NA) and beneficial effects of Tyr on cognition may be mediated by increases in either DA or NA, or both (Jongkees et al., 2015). For this reason we cannot definitively conclude that the findings reported in the present study are mediated solely by DA and not by NA, in particular as pharmacological enhancement of NA has recently been shown to modulate tDCS-induced plasticity (Kuo et al., 2016). There is evidence that in particular DA modulates the effect of tDCS, as DA antagonists can abolish the effect of tDCS on cortical excitability (Nitsche et al., 2006), but more research is necessary before we could conclude the effects of Tyr on tDCS are mediated solely by DA.

Lastly, it is important to consider that our results may have depended significantly on our choice of stimulation parameters, such as location of the return electrode, size of the electrodes, applying stimulation before or during the task of interest, the current intensity, and stimulation duration. tDCS studies so far have used many different parameters, which may play an under-investigated role in determining the efficacy of the stimulation (see Woods et al., 2016). This highlights the possibility our results could have been different had we chosen different parameters, and future research should aim to systematically investigate whether the influence of DA on tDCS might depend on these parameters to produce different cognitive-behavioral outcomes.

Although more research is needed, the present study provides first evidence that Tyr administration modulates the cognitive-behavioral effects of tDCS and, in doing so, tentatively supports the hypothesis that DA plays a causal role in prefrontal tDCS. Despite probably being only one of many important factors, we recommend future studies to consider the effects of DA on tDCS in order to achieve more consistent and replicable results.