Neuromodulation of cognitive-behavioral control

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Chapter Seven

The COMT Val^{158}Met polymorphism does not modulate the after-effect of tDCS on working memory

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Abstract

Although transcranial direct current stimulation (tDCS) can alter cortical excitability, neural plasticity and cognitive-behavioral performance, its effects are known to vary across studies. A partial account of this variability relates to individual differences in dopamine function. Indeed, dopaminergic manipulations alter the physiological and cognitive-behavioral effects of tDCS, and genetic polymorphisms related to dopamine have predicted individual response to online tDCS (i.e., stimulation overlapping with the critical task). Notably, the role of individual differences in dopamine has not yet been properly assessed in the effect of offline tDCS (i.e., stimulation prior to the critical task). Therefore, we investigated if and how the COMT Val^{158}Met polymorphism (rs4680) modulates the after-effect of prefrontal tDCS on verbal working memory (WM). 139 participants were genotyped for the COMT Val^{158}Met polymorphism and received anodal-over-left, cathodal-over-right (AL-CR) dorsolateral prefrontal cortex stimulation, cathodal-over-left, anodal-over-right (CL-AR) or sham stimulation in a between-subjects, pretest-posttest study design. WM was assessed using the N-back task. The results provide no evidence that the COMT polymorphism impacts the after-effect of prefrontal tDCS on WM. Taken together with previous findings on the interaction between dopamine and tDCS effects, the results of the present study suggest that (i) dopamine might differentially impact online and offline effects of tDCS, and (ii) findings from studies including pharmacological manipulation should be generalized only with caution to findings of inter-individual differences. Specifically, state (i.e., a manipulation of) and trait (i.e., baseline) differences in dopamine appear to exert different effects on tDCS.

Introduction

Current research has increasingly focused on the idea that non-invasive brain stimulation can serve as an effective tool to investigate and possibly enhance the neuromodulation of cognitive-behavioral performance. Of the available techniques, transcranial direct current stimulation (tDCS) is a popular method of transiently enhancing performance or augmenting the gains from extended training. tDCS alters cortical excitability (Nitsche & Paulus, 2000) and, at longer stimulation periods, affects neural plasticity (Nitsche & Paulus, 2001; Nitsche, Nitsche, et al., 2003) by inducing a polarity-dependent shift in the resting membrane potential of cortical neurons. It has been questioned whether these physiological changes translate to reliable effects on cognition (Horvath et al., 2015a, 2015b; Mancuso, Ilieva, Hamilton, & Farah, 2016), but reviews on this issue often suffer many limitations that prevent an unequivocal answer (Antal et al., 2015). Notwithstanding the variability in results that might be explained by methodological differences across studies, it has been suggested that individual differences in dopamine (DA) function within and across studies might partially account for variable effects of tDCS (Li et al., 2015; Wiegand et al., 2016). In the present study we explore this idea by investigating whether a genetic predisposition towards higher or lower prefrontal DA activity predicts individual differences in the effect of tDCS on verbal working memory (WM).

There is converging evidence DA indeed has an important impact on tDCS effects. Pharmacological stimulation of DA receptors has non-linear effects on tDCS-induced neuroplasticity, and blockage of DA receptors can eliminate effects on plasticity entirely (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). These studies point to an inverted-U-shaped relationship between DA activity and tDCS effects (Wiegand et al., 2016), as low and high, but not moderate, stimulation of DA receptors abolished tDCS-induced changes in neuroplasticity (Fresnoza, Paulus, et al., 2014; Monte-Silva et al., 2010), whereas moderate DA enhancement strengthened long-term depression (LTD)-like effects of

cathodal tDCS, while it converted after-effects of anodal tDCS from long-term potentiation (LTP)- to LTD-like effects (Kuo et al., 2008; Monte-Silva et al., 2010). An inverted-U-shaped relationship is also observed in studies of pre-existing differences rather than artificially-induced changes in DA function, with results varying depending on the type of stimulation and experimental task conditions. Using the COMT Val^{158}Met polymorphism to estimate individual differences in prefrontal DA, it was shown tDCS impaired cognitive flexibility in individuals with high DA activity who received excitatory stimulation during task performance (Plewnia et al., 2013). In contrast, tDCS impaired response inhibition in individuals with low DA activity who received inhibitory stimulation (Nieratschker et al., 2015).

These results were mirrored in a recent study examining the effect of a modest dopaminergic manipulation on the cognitive-behavioral rather than the physiological effect of tDCS (Jongkees, Sellaro, et al., 2017). This was done by combining tDCS with administration of L-tyrosine, the biochemical precursor of L-dopa and DA, to transiently enhance DA activity. Specifically, it was shown that prefrontal tDCS impaired WM performance on the N-back task when L-tyrosine was combined with excitatory stimulation of the left dorsolateral prefrontal cortex (DLPFC), yet it trend-wise enhanced performance when L-tyrosine was combined with inhibitory stimulation of the left DLPFC. The authors speculated that DA and tDCS might interact on cortical excitability, with increased DA combined with excitatory stimulation resulting in overexcitability of the cortex whereas combined with inhibitory stimulation it might serve to promote cortical signal-to-noise ratio. Together with the studies on the COMT polymorphism, these findings highlight a state-dependency of tDCS effects, with the type of stimulation interacting with the dopaminergic activity state.

To account for these behavioral findings, it has been proposed that tDCS might bring an individual closer to or further away from an optimal level of dopaminergic signaling (Nieratschker et al., 2015; Plewnia et al., 2013; Wiegand et al., 2016), which would be consistent with animal literature demonstrating tDCS can enhance DA release (Tanaka et al., 2013).

Specifically, individuals with an already optimal level of signaling, such as those with high prefrontal DA activity due to genetic predisposition or L-tyrosine administration, might be pushed towards a suboptimal, too high level of activity that results in impaired performance when receiving excitatory stimulation. Conversely, individuals with a lower-than-optimal level of signaling due to low prefrontal DA activity might show impaired performance when that activity is further reduced by inhibitory stimulation. In brief, an individual's initial position on the inverted-U curve relating DA and performance would determine whether a shift toward the right or left on the curve (due to excitatory or inhibitory stimulation, respectively) enhances or impairs performance. It should be noted that this interaction between tDCS and DA might not necessarily reflect a direct impact of the former on the latter, but instead be mediated by tDCS-induced changes in levels of glutamate and GABA (Bachtiar et al., 2015; Soyoung Kim et al., 2014; Stagg et al., 2009).

The present study

The line of reasoning presented above has been primarily applied to online effects of tDCS, i.e., stimulation *overlapping* with the critical task. In the present study we investigated whether this hypothesis extends to offline tDCS as well, i.e., stimulation *prior* to the critical task. Whereas online effects of tDCS are attributed mainly to a modulation of cortical excitability, offline effects of tDCS reflect changes in neural plasticity (Nitsche & Paulus, 2000; Nitsche, Nitsche, et al., 2003). Both can be sensitive to DA, with the interaction between DA and online tDCS being mediated partially by interacting effects on task-induced activity (Bortoletto et al., 2015; Mattay et al., 2003), whereas the interaction with offline tDCS might be mediated by effects on N-methyl-D-aspartate (NMDA) receptors which drive neuroplasticity via long-term potentiation and depression (Gurden et al., 2000; Huang et al., 2004; Spencer & Murphy, 2000). Considering a DA manipulation altered the cognitive-behavioral after-effect of tDCS (Jongkees, Sellaro, et al., 2017) and individual baseline differences in DA have predicted online effects of tDCS (Nieratschker et al., 2015; Plewnia et al., 2013), it is conceivable these individual differences

predict the after-effects of offline tDCS as well. We were interested in the effects on WM in particular, because this cognitive function is the most-often investigated function in tDCS studies. Hence a demonstration or a lack of an impact of individual differences in DA on tDCS after-effects on WM would have implications for a majority of the existing tDCS literature.

Following the only two available studies on individual differences in DA and cognitive-behavioral effects of prefrontal tDCS (Nieratschker et al., 2015; Plewnia et al., 2013), we assessed genetic predisposition toward higher or lower dopaminergic signaling in the prefrontal cortex using the COMT Val^{158}Met polymorphism. The COMT enzyme is responsible for degradation of extracellular DA, and differences in thermolability of the enzyme determined by different COMT polymorphisms affect the rate at which DA is degraded (Weinshilboum, Otterness, & Szumlanski, 1999). Carriers of the Val allele have a less thermolabile enzyme that results in faster degradation and, hence, lower concentrations of DA, whereas carriers of the Met allele have a more thermolabile enzyme that results in slower degradation and, hence, higher concentrations of DA. The COMT polymorphism relates to prefrontal DA activity in particular (Karoum, Chrapusta, & Egan, 1994) due to a relative lack of DA transporters in the prefrontal cortex (PFC) as compared to their abundance in the striatum (Lewis et al., 2001). Consistent with a lower prefrontal DA concentration, Val carriers demonstrate less efficient cortical processing (Egan et al., 2001; Mattay et al., 2003) and worse behavioral performance during WM tasks (Goldberg et al., 2003), but also better task-switching performance as compared to Met carriers (Colzato, Waszak, Nieuwenhuis, Posthuma, & Hommel, 2010). Most important for our purposes, this polymorphism has previously predicted the effect of prefrontal tDCS on cognitive-behavioral performance (Nieratschker et al., 2015; Plewnia et al., 2013), making it the most obvious marker of individual differences in DA for the present purpose.

Considering tDCS effects likely vary depending on experimental parameters such as electrode placement and stimulation duration, we opted for a stimulation montage and duration of which the after-effects are sensitive to

a mild DA manipulation (Jongkees, Sellaro, et al., 2017). Electrodes were placed over DLPFC in a bilateral bipolar-balanced montage (Nasseri et al., 2015). This montage enhanced WM in antidepressant-free patients with major depressive disorder (Oliveira et al., 2013) and, more importantly, interacted in healthy adults with a dopaminergic manipulation on WM (Jongkees, Sellaro, et al., 2017) in a manner similar to studies on individual differences in DA and cognitive-behavioral effects of tDCS (Nieratschker et al., 2015; Plewnia et al., 2013).

In brief, 139 participants were genotyped for the COMT Val^{158}Met polymorphism and received either anodal-over-left, cathodal-over-right (AL-CR) DLPFC stimulation, cathodal-over-left, anodal-over-right (CL-AR) or sham stimulation in a between-subjects, sham-controlled, pretest-posttest study design. Based on previous findings (Jongkees, Sellaro, et al., 2017; Nieratschker et al., 2015; Plewnia et al., 2013), as compared to sham stimulation, we expected individuals with high dopaminergic signaling, i.e., Met carriers, to demonstrate worse WM performance after receiving excitatory stimulation (AL-CR) over the left DLPFC, whereas individuals with low dopaminergic signaling, i.e., Val carriers, were expected to demonstrate worse WM performance after receiving inhibitory stimulation (CL-AR) over the left DLPFC. The inverted-U-curve proposed by (Wiegand et al., 2016) also suggests that Val carriers potentially benefit behaviorally from a slight increase in dopaminergic signaling due to excitatory stimulation (i.e., being shifted right and upwards on the inverted-U-curve). Notwithstanding these hypothesized findings, it is important to consider that pharmacological manipulations do not necessarily mimic the effects of natural variation in a neurotransmitter system (Boy et al., 2011), pointing to the possibility that COMT-tDCS interactions do not necessarily mirror the interaction between dopaminergic manipulations and tDCS. This is a significant possibility in light of the fact that no published study has yet demonstrated a role for individual differences in DA in the after-effects of tDCS on WM. This suggests DA-tDCS interactions might vary or not apply to every type of stimulation and/or experimental task, as our results will indeed indicate.

Material and methods

Participants

139 right-handed undergraduate students participated in a study on tDCS and memory. Participants were randomly assigned to one of the three stimulation types (AL-CR, CL-AR, or sham). 9 participants were identified as performance outliers as described in the Results section, leaving a total of 130 participants for further analysis. The resulting groups did not differ with respect to age, $F(4,121) = .61$, $p = .656$, or gender distribution, $X^2(4, N = 130) = 1.06$, $p = .901$, see Table 1 for group demographics. All participants were screened individually using the Mini International Neuropsychiatric Interview (MINI), a short, structured interview of approximately 15 min that screens for several psychiatric disorders and drug use (Sheehan et al., 1998), and has been used previously in neuromodulation research, including research on L-tyrosine and tDCS (Jongkees, Immink, & Colzato, 2017; Jongkees, Sellaro, et al., 2017). Participants were included if they met the following criteria: (i) between 18 and 30 years; (ii) no history of neurological or psychiatric disorders; (iii) no history of substance abuse or dependence; (iv) no chronic or acute medication; (v) no implants such as pacemakers or any kind of metal in the body, nor any skin conditions, for safety reasons concerning tDCS. One exception was hormonal contraceptive use in females, which was required to limit fluctuations in hormone levels that can influence DA function and confound group differences (Colzato & Hommel, 2014; Czoty et al., 2009; Jacobs & Esposito, 2011). All participants met these criteria. Before the study, participants were informed of the procedure and potential side-effects of tDCS (i.e., itching, stinging or burning sensation from the electrodes, reddening of the skin and headache). None of the participants reported major side-effects. The study conformed to the ethical standards of the declaration of Helsinki and the protocol was approved by the local ethical committee (Leiden University, Institute for Psychological Research).

Table 1. Group demographics

	AL-CR	CL-AR	Sham
N			
Met/Met	16	11	13
Val/Met	20	21	19
Val/Val	12	8	10
Gender F:M			
Met/Met	9:7	7:4	9:4
Val/Met	15:5	11:10	15:4
Val/Val	8:4	6:2	8:2
Age in years			
Met/Met	21.1 (3.1)	22.1 (2.3)	21.5 (2.8)
Val/Met	22.4 (2.9)	21.3 (2.7)	21.4 (2.5)
Val/Val	22.5 (2.9)	23.1 (3.9)	22.7 (3.2)

Genotyping

Genetic material to determine COMT genotype was collected using buccal swabs, which were analyzed by the company BaseClear (The Netherlands). The SNP Val158Met of the COMT gene (rs4680) was genotyped using Applied Biosystems (AB) TaqMan technology. All genotypes were scored by two independent readers by comparison to sequence-verified standards. For COMT Val158Met three genotype groups were established: Val/Val homozygotes, Val/Met heterozygotes and Met/Met homozygotes. COMT genotype was available in all participants.

Genotype distribution for COMT Val^{158}Met polymorphism in our Dutch healthy population was 30 Val/Val homozygous subjects (23.08%), 60 Val/Met heterozygous subjects (46.15%) and 40 Met/Met homozygous subjects (30.77%). All resulting genotype frequencies from our cohort of participants did not deviate from Hardy-Weinberg equilibrium (p = .415). No significant differences were found among genotype frequencies with respect to age, $F(2,127) = 1.85$, $p = .161$ or gender distribution, $X^2(2, N = 130) = .94$, $p = .625$.

N-back task

WM performance was assessed using the N-back task (Kane et al., 2007), which is predominantly used in tDCS studies on 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 et al., 2011). As in the study on L-tyrosine and tDCS (Jongkees, Sellaro, et al., 2017), a letter-based N-back task was used to assess verbal WM (Colzato, Jongkees, et al., 2013). To prevent potential ceiling-effects induced by repeated practice in a pretest-posttest design, a 2-back and 4-back condition was included in each pretest and posttest.

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 for targets (i.e., repetition) and non-targets (i.e., non-repetition), respectively. Mapping of response buttons to targets and non-targets was not counterbalanced across participants to prevent differences in response mapping across genotypes. After an incorrect or belated response (latency longer than 1000 ms) a brief tone was presented to signal the error. Both the 2-back and the 4-back conditions consisted of two blocks of 51 + N trials. For example, a 2-back block consisted of 53 trials. Regardless of the load condition, each block comprised 21 targets and 30 non-targets. All participants performed the 2-back condition first and then the 4-back condition, and each N-back condition was preceded by 17 + N practice trials (7 targets and 10 targets).

Transcranial direct current stimulation

In line with (Jongkees, Sellaro, et al., 2017), two electrodes of 35 cm^2 (5 cm x 7 cm) were placed over DLPFC in a bilateral bipolar-balanced montage (Nasseri et al., 2015), i.e., in symmetrical positions. For each individual participant the DLPFC was located using the international 10/20 system for placing electrodes on the scalp (Jasper, 1958). As such, for the AL-CR montage the anode and cathode were placed over F3 and F4, respectively, whereas this placement was reversed for the CL-AR montage. In the sham condition, half of participants received the AL-CR montage and the other half received the CL-AR montage.

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. Sham stimulation was exactly the same but lasted for 15 s instead of 15 min, thus providing a similar initial sensation as active stimulation. The after-effects of 15 min of tDCS typically last 30 to 60 min, whereas stimulation of only a few seconds produces no changes in cortical excitability or plasticity (Nitsche et al., 2008).

The experience of side-effects due to tDCS was assessed through self-report ratings for the following symptoms: (i) headache, (ii) neck pain, (iii) nausea, (iv) muscle contractions in the face or neck, (v) stinging sensation under the electrodes, (vi) burning sensation under the electrodes, and (vii) a nonspecific, uncomfortable feeling. Consistent with previous studies, the most prominent side-effects were stinging and burning sensations under the electrodes (Bikson et al., 2009), although no participants voiced major complaints.

Procedure

Participants gave written consent upon entering the lab. After filling in a questionnaire assessing their general health, they completed a pretest of the N-back task, which took on average 20 min. Subsequently the tDCS montage was mounted on the participants' scalp and stimulation was started. During the 15 min of stimulation, participants gave buccal swabs to determine COMT genotype. Following stimulation, the tDCS electrodes were removed and participants completed the posttest of the N-back, which was identical in structure to the pretest and took on average 20 min. In total the procedure took approximately 90 min.

Statistical analysis

Aside from parameters such as hit rate and correct rejections, we were interested in target sensitivity, indexed by d' prime derived from signal

detection theory (Swets et al., 1961). This measure combines hit rate and false alarms to provide an index of the ability to discriminate targets from non-targets, with higher scores indicating more selective and correct reporting of targets. d' prime was calculated, and perfect scores were corrected for, as described earlier (Colzato, Jongkees, et al., 2013).

First, each group was checked for outlier performance (below or above 3 standard deviations of the group mean) on d' prime, hit rate, correct rejections and reaction time (RT). Subsequently, a repeated measures analysis of variance (rmANOVA) was conducted with time (pretest vs posttest) and WM load (2-back vs 4-back) as within-subject factors and type of stimulation (AL-CR vs CL-AR vs sham) and COMT genotype (Val/Val vs Val/Met vs Met/Met) as between-subject factors. Separate analyses were performed for d' prime, hit rate, correct rejections and RT for targets and non-targets.

Results

4 participants were identified as outliers based on either pretest or posttest d' prime scores, additional 3 participants were identified as outliers based on hit rate or correct rejections, and another 2 participants were identified as outliers based on RT. This left a total of 130 participants for subsequent analyses. See Table 2 for an overview of group scores on the N-back, and see Figure 1 for a depiction of the d' prime scores.

None of the dependent variables (d' prime, hit rate, correct rejections and RT) demonstrated a main effect of stimulation ($ps \geq .406$), an interaction between time and stimulation ($ps \geq .494$), nor a three-way interaction involving load ($ps \geq .252$), suggesting that tDCS did not modulate N-back performance when disregarding COMT genotype. Only RT to non-targets revealed a main effect of COMT, $F(2,121) = 3.43$, $p = .036$, partial $\eta^2 = .054$, with Val homozygotes demonstrating higher RT than Met homozygotes ($M = 591$ vs 557 ms, $p = .012$) but not Val/Met heterozygotes ($M = 577$ ms, $p = .286$), nor was there a significant difference between Met homozygotes and heterozygotes ($p = .068$). All other measures revealed no main effect of COMT

(*p*s ≥ .140), nor an interaction with time (*p*s ≥ .465) or a three-way interaction involving load (*p*s ≥ .211).

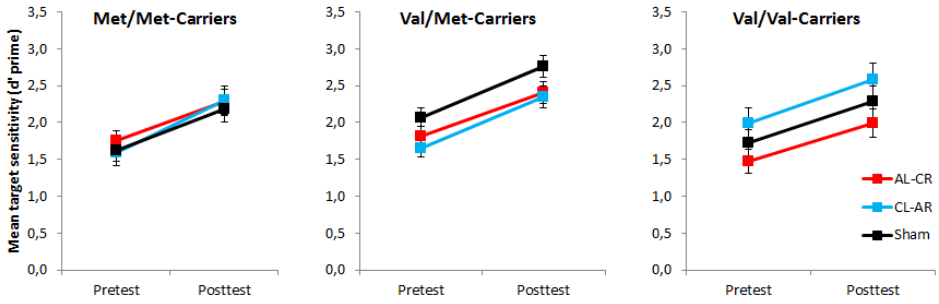

Figure 1. D' prime scores as a function of time (pretest vs posttest), stimulation (AL-CR vs CL-AR vs Sham), and COMT genotype (Met/Met vs Val/Met vs Val/Val).

Most important to the present study, no dependent measures demonstrated a significant three-way interaction between time, stimulation and COMT (*p*s ≥ .476), nor a four-way interaction involving load (*p*s ≥ .505) except for RT to targets $F(4, 121) = 2.67$, $p = .036$, partial $\eta^2 = .054$. To disentangle this four-way interaction we first computed individual difference scores for pretest and posttest RT and then separately submitted 2-back and 4-back scores to the ANOVA with stimulation and genotype as between-subject factors. This revealed no significant interaction between stimulation and COMT for either the 2-back, $F(4,121) = 1.53$, $p = .198$, or the 4-back, $F(4,121) = 1.03$, $p = .394$.

To obtain further evidence for a lack of an impact of COMT on tDCS effects and WM performance, we performed post-hoc comparisons using non-parametric Mann-Whitney's U tests for the 2 main hypotheses. Specifically, previous studies predicted Met homozygotes would demonstrate impaired performance following AL-CR stimulation as compared to sham, whereas Val homozygotes would become impaired following CL-AR stimulation as compared to sham. Difference scores for pretest and posttest for each dependent variable were computed separately for the 2-back and 4-back, but none of the comparisons demonstrated significant stimulation group

differences, *p*s ≥ .326. As such, the results do not point towards a modulation of tDCS after-effects on WM by the COMT genotype.

Table 2. N-back scores

	AL-CR		CL-AR		Sham	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
2-back						
d' prime						
Met/Met	1.96 (.51)	2.39 (.49)	1.81 (1.14)	2.65 (.97)	1.73 (.38)	2.36 (.68)
Val/Met	1.98 (.61)	2.55 (.71)	1.78 (.59)	2.42 (.79)	2.24 (.80)	2.88 (.98)
Val/Val	1.72 (.62)	2.30 (.55)	2.26 (.72)	2.87 (.53)	1.83 (.50)	2.30 (.81)
Hit rate in %						
Met/Met	84.1 (9.4)	89.9 (6.1)	79.9 (13.4)	91.1 (8.8)	83.2 (8.5)	91.2 (5.6)
Val/Met	86.1 (8.4)	91.8 (8.9)	83.8 (7.8)	90.4 (7.1)	88.5 (10.0)	92.6 (7.4)
Val/Val	80.6 (9.7)	88.7 (3.8)	89.9 (6.1)	96.7 (2.2)	86.2 (7.1)	89.5 (8.5)
Correct reject. in %						
Met/Met	80.5 (5.2)	83.7 (6.6)	76.2 (15.3)	83.8 (10.7)	75.5 (6.3)	79.5 (11.4)
Val/Met	78.6 (8.4)	82.5 (7.9)	75.5 (11.1)	82.0 (12.0)	78.7 (12.3)	86.0 (11.8)
Val/Val	77.8 (11.3)	83.2 (9.9)	80.0 (9.4)	81.9 (11.2)	74.2 (8.6)	80.3 (11.4)
RT_{Target} *in ms*						
Met/Met	598 (75)	554 (76)	583 (48)	550 (57)	589 (53)	548 (52)
Val/Met	610 (51)	589 (57)	593 (73)	568 (67)	613 (73)	593 (54)
Val/Val	615 (50)	591 (73)	626 (73)	601 (69)	618 (55)	600 (75)
$RT_{Non\text{-}target}$ *in ms*						
Met/Met	558 (85)	502 (71)	560 (95)	495 (72)	526 (75)	482 (79)
Val/Met	543 (94)	480 (81)	545 (73)	499 (64)	506 (51)	458 (61)
Val/Val	522 (59)	495 (73)	535 (82)	461 (60)	540 (86)	486 (77)
4-back						
d' prime						
Met/Met	1.55 (.87)	2.17 (.70)	1.37 (.88)	1.96 (.91)	1.53 (.61)	2.02 (.63)
Val/Met	1.65 (.58)	2.26 (.60)	1.52 (.54)	2.27 (.81)	1.90 (.58)	2.64 (.80)
Val/Val	1.22 (.37)	1.69 (.55)	1.72 (.28)	2.28 (.42)	1.62 (.56)	2.26 (.62)
Hit rate in %						
Met/Met	57.9 (17.0)	65.6 (14.8)	54.8 (16.6)	57.6 (18.8)	57.3 (14.0)	64.1 (15.2)
Val/Met	58.5 (11.4)	64.3 (12.9)	60.8 (12.7)	65.4 (17.9)	62.8 (11.9)	71.8 (14.2)
Val/Val	54.0 (13.2)	63.3 (19.1)	57.4 (11.8)	63.7 (9.8)	56.4 (12.1)	63.1 (14.7)
Correct reject. in %						
Met/Met	89.1 (7.6)	94.5 (5.3)	86.4 (11.5)	93.9 (7.0)	89.0 (7.9)	94.0 (4.1)
Val/Met	90.3 (7.1)	95.8 (4.7)	87.5 (7.1)	95.6 (3.3)	92.5 (5.5)	96.5 (5.0)
Val/Val	85.4 (8.4)	89.0 (5.4)	92.5 (4.9)	96.7 (3.1)	91.7 (4.7)	96.7 (2.2)
RT_{Target} *in ms*						
Met/Met	595 (72)	573 (43)	616 (81)	589 (94)	605 (79)	567 (64)
Val/Met	601 (70)	572 (91)	623 (100)	563 (108)	575 (53)	531 (62)
Val/Val	593 (61)	524 (63)	583 (61)	541 (47)	600 (43)	542 (66)
$RT_{Non\text{-}target}$ *in ms*						
Met/Met	588 (83)	528 (78)	566 (69)	524 (76)	551 (74)	504 (74)
Val/Met	578 (62)	533 (56)	583 (51)	529 (61)	596 (69)	548 (67)
Val/Val	570 (79)	524 (72)	622 (23)	563 (68)	610 (72)	552 (78)

Average N-back scores with standard deviation in parentheses

Discussion

The present study investigated whether the after-effect of prefrontal tDCS is modulated by individual differences in DA function. To this end participants were genotyped for the COMT Val^{158}Met polymorphism to estimate prefrontal DA activity and completed tests of WM performance before and after tDCS over the DLPFC. Although a mild DA manipulation previously modulated the after-effect of tDCS on WM (Jongkees, Sellaro, et al., 2017), the current results indicate this effect does not extend to pre-existing differences in, rather than a manipulation of DA activity. Although the result contrasts with two previous studies on COMT genotype and online effects of prefrontal tDCS on behavioral performance (Nieratschker et al., 2015; Plewnia et al., 2013), this does not undermine the results from previous studies. Instead, our results add to them by suggesting two important implications for future studies on tDCS.

First, whereas previous studies looked at an interaction between COMT and *online* effects of tDCS (i.e., stimulation overlapping with the critical task), the present study examined *offline* effects of tDCS (i.e., stimulation *prior* to the critical task). Online effects of tDCS are likely to reflect transient changes in cortical excitability (Nitsche & Paulus, 2000), whereas offline effects of tDCS are related to changes in synaptic plasticity (Nitsche & Paulus, 2001; Nitsche, Nitsche, et al., 2003). As such, the current results combined with previous findings indicate that the COMT genotype might differentially affect tDCS-induced changes in cortical excitability and neural plasticity. Although the present study implies this distinction exclusively at a behavioral level of results, future studies might investigate whether online and offline effects on physiology are also differentially affected by COMT genotype. Such a distinction would notably contrast with the glutamatergic and GABAergic systems, which instead have been shown to be relevant for the offline but not online effects of tDCS (Nitsche, Fricke, et al., 2003).

Second, the results underscore a need for caution when generalizing results from pharmacological manipulation of a neurotransmitter system to results from pre-existing baseline differences in that system. Whereas administration of DA's precursor L-tyrosine did modulate the after-effect of

prefrontal tDCS on WM (Jongkees, Sellaro, et al., 2017), this pattern of results was not mirrored by the COMT genotype as shown in the present study. Although it is possible that similar effects are observable on a physiological level, e.g., the directionality of change in cortical excitability, the impact of genetic predisposition might not have been large enough to immediately produce detectable differences at the behavioral level. On the one hand, this might be explained by the possibility that pharmacological manipulation induces larger changes in a neurotransmitter system that more easily cross a threshold at which behavioral changes are observed. As such, it might be that the smaller effect of COMT genotype requires longer periods of stimulation, repeated stimulation and large sample sizes to become apparent. On the other hand, it is possible that manipulation of a neurotransmitter system exerts different physiological and behavioral effects than naturally-occurring variation in that system (Boy et al., 2011), leading to different interactions with the psychophysiology of tDCS.

Notably, in neither this study nor the study on L-tyrosine (Jongkees, Sellaro, et al., 2017) did tDCS have a main effect on WM. Although this might be taken as evidence against the efficacy of tDCS in enhancing cognition, it is important to consider the possibility that tDCS effects can require several sessions to become behaviorally observable, possibly strengthening the consolidation of practice between sessions (Au et al., 2016; Au, Karsten, Buschkuehl, & Jaeggi, 2017). More importantly for the interpretation of the present study, L-tyrosine was shown to modulate the effect of single-session tDCS whereas COMT genotype did not as shown here. In light of the possibility that COMT effects might be smaller than pharmacological manipulation of DA, future studies could examine whether COMT genotype does predict effects of tDCS following multiple sessions of stimulation, and as mentioned before, whether these effects are different for online and offline tDCS (Mancuso et al., 2016).

Regardless of the exact underlying mechanism, the differential effect of L-tyrosine and COMT on tDCS after-effects on WM cannot be attributed to methodological differences between studies such as type of montage or

duration of stimulation, which were identical in both studies (Jongkees, Sellaro, et al., 2017). One notable difference is that the present study includes a pretest of WM performance, which might have produced a learning effect that obscured tDCS-induced changes in performance and its interaction with COMT. Although a pretest was necessary to exclude the possibility that results were driven by baseline differences due to COMT genotype, the present study cannot definitively rule out that a learning effect accounts for the different results across studies. One method of alleviating this issue in future studies might be to use adaptive N-back tasks (Au et al., 2016; Jaeggi et al., 2014), which potentially lessen the obscuring effect of practice in static N-back tasks.

To conclude, the present study demonstrates no impact of COMT genotype on the impact of the after-effect of single-session prefrontal tDCS on WM. In doing so, this study indicates that (i) DA might differentially modulate the effects of online and offline tDCS, and (ii) more generally, tDCS results obtained in pharmacological studies should be generalized with caution to studies of individual differences in neurotransmitter function.