

Cognition and Behavior

# No Effect of 2 mA Anodal tDCS Over the M1 on Performance and Practice Effect on Grooved Pegboard Test and Trail Making Test B<sup>1,2,3</sup>

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## Abstract

Previous studies suggest that transcranial direct current stimulation (tDCS) can facilitate motor performance and learning. In this double-blind experiment, 60 healthy human subjects (29 females) were randomized into three groups (active tDCS, sham tDCS, and no-treatment control group) in order to investigate the effect of a 20 min session of 2 mA tDCS over the motor cortex contralateral to the dominant hand on practice effect and performance on the Grooved Pegboard Test (GPT) and Trail Making Test (TMT). Performance was operationalized as the time to complete the tests before, during, and after stimulation. The practice effect was termed as the difference in time to complete the tests from pretest to post-test. Data on body mass index (BMI), head circumference, sleep status, interelectrode impedance, and caffeine and nicotine use were sampled to control for the influence of individual differences on the effect of tDCS. Adverse effects were registered using a standardized form. The results indicated no effect of tDCS on performance and practice effects on the GPT and TMT. For all groups, BMI was a predictor for a practice effect on the TMT. In the active tDCS group, high caffeine intake and low impedance predicted a practice effect on the GPT for the dominant hand. The present results suggest that impedance levels in tDCS studies should be routinely reported in future studies, as it might not only provide valuable information on the efficacy of the blinding conditions and participant discomfort, but also correlate with individual differences that are relevant to the outcome of the stimulation.

*Key words:* cognition; motor speed; transcranial direct current stimulation

## Significance Statement

Transcranial direct current stimulation (tDCS) is a noninvasive brain stimulation technique that can modulate neuronal activation thresholds. The capability to enhance or diminish cortical excitability has previously been consistently demonstrated in experiments, with no serious adverse effects. The effect of tDCS on behavioral outcomes, especially on healthy subjects, appears less consistent. We tested whether stimulation over the motor cortex affected performance and practice effect on two commonly used neuropsychological tests that require fine motor skills, finger dexterity, and psychomotor speed. The results indicated no effect of the stimulation on these outcomes. Uniquely for subjects who received active tDCS, caffeine intake and electrode impedance predicted increased practice effect. The authors suggest that impedance levels should be routinely reported in tDCS studies.

## Introduction

Plasticity in the motor cortex (M1) plays an important role in motor skill learning (Ungerleider, 1995), and it is likely that a neurophysiological correlate of motor skills resides in the M1 (Nudo et al., 1996). As the acquisition of new motor skills are accompanied by changes in neuronal activity and excitability (Nitsche et al., 2003), it is therefore possible that transcranial direct current stimulation (tDCS) of the M1 can induce measurable changes in motor learning and performance. Results from several studies do indeed indicate that stimulation can enhance performance on motor tasks in healthy subjects. For instance, anodal tDCS over the left motor area improves right hand performance on a finger-sequencing task more than cathodal stimulation, but with opposite polarity effects for the left hand (Vines et al., 2006). Anodal tDCS over the M1 can also facilitate implicit learning on a serial reaction time task (Nitsche et al., 2003). In addition to polarity-specific effects, the timing of stimulation seems to be important, as anodal, but not cathodal, tDCS during an explicit learning task increases the rate of learning, but if applied prior to the learning task, both cathodal and anodal tDCS decrease the rate of learning (Stagg et al., 2011). In aged healthy subjects, anodal tDCS over the left M1 significantly improves right hand performance, compared to sham tDCS, on a task that mimics activities of daily living, including fine motor skills (Hummel et al., 2010). Furthermore, in older subjects, recent evidence indicates that anodal tDCS increases the retention of practice effect, compared to sham tDCS, on the grooved pegboard test (GPT) 35 min after the stimulation, but with no difference immediately following the stimulation (Parikh and Cole, 2014). In stroke patients, tDCS improves performance both on simple (Hummel et al., 2005) and more complex motor tasks (Fregni et al., 2005; Boggio et al., 2007). The variation in methodology makes the above results difficult to compare. However, a meta-analysis on polarity-specific effects of tDCS on behavioral motor and cognitive outcomes (Jacobson et al., 2012) indicate that the facilitation effect of anodal tDCS is more consistent than the inhibition effect of cathodal tDCS in studies with cognitive outcomes. Furthermore, results from another study (Cuypers et al., 2013) suggest that the effect sizes of anodal tDCS facilitation effects on learning tasks can

be increased by increasing the stimulation intensity. Finally, there is also a high intersubject variability in the response to tDCS (Wiethoff et al., 2014). Participant age, interindividual differences in anatomy (Datta et al., 2012), and functional state of the brain, such as fatigue and activity (Silvanto et al., 2008; Antal et al., 2014), might affect the outcome of the stimulation. These factors are often not considered in studies that investigate the effect of tDCS on motor outcomes.

The aim of the present study is to test the effect of 2 mA anodal tDCS in healthy subjects. The present study tests performance and practice effects on two commonly used neuropsychological tests, which measure fine motor skills (GPT) and psychomotor speed [trail making test (TMT)] in an experimentally controlled study. In addition to a sham group, the present study included a no-treatment control group to estimate possible placebo effects (Benedetti et al., 2003), as the true placebo effect is represented by the difference between the sham and no treatment conditions (Fields and Levine, 1984).

It was hypothesized that the time to complete the tests would be lower during stimulation in the group that received active tDCS, compared to the sham tDCS and control groups. Furthermore, it was hypothesized that the practice effect, operationalized as the reduction in time to complete tests from pretest to post-test would be largest in the active tDCS group. Additionally, in order to investigate the influence of overt interindividual differences, body mass index (BMI) and head circumference were measured. The investigation correlates the functional state of the brain, sleep status, caffeine use, and nicotine use. Finally, electrode impedance was investigated as a possible predictor for practice effects in the active tDCS group.

## Materials and Methods

### Participants

Sixty healthy human subjects (29 females; demographic properties are displayed in Table 1) participated in the study. All participants were informed that the study investigated the effect of tDCS on fine motor ability. The exclusion criteria included severe psychiatric conditions, defined as bipolar disorder, severe depression, and schizophrenia. Additional exclusion criteria consisted of neurological conditions, developmental disorders, pregnancy, and drug abuse. No participants reported that they were receiving treatment with a medication that acted on the CNS. No subjects who declared interest in participating met the exclusion criteria. The study was approved by the Regional Committee for Medical and Health Research Ethics (2010/2256), and all participants gave their written informed consent in accordance with the Declaration of Helsinki guidelines.

### Study design

The study was designed as a double-blind study (single-blind study for the control group) with the following three groups: active tDCS group, sham tDCS group, and natural history control group with three repeated mea-

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**Table 1: Mean values for demographic, head circumference, and behavioral measures**

	Sham group	Active group	Control group	<i>p</i> *
Total <i>n</i> (female <i>n</i> )	20 (10)	20 (11)	20 (8)	0.64
Right handed ( <i>n</i> )	19	19	18	0.77
Age (years)	23.65 (3.12)	24.10 (4.24)	23.80 (3.33)	0.92
BMI (kg/m <sup>2</sup> )	25.01 (4.10)	24.32 (2.89)	25.80 (4.55)	0.49
Head circumference (cm)	57.30 (2.01)	57.30 (1.47)	57.48 (1.61)	0.94
Sleep time (h)	6.95 (1.06)	6.95 (1.38)	6.78 (1.63)	0.90
Doses of nicotine ( <i>n</i> )	0.65 (1.27)	0.60 (.88)	1.5 (2.01)	0.10
Cups of coffee ( <i>n</i> )	0.60 (.82)	0.75 (1.29)	0.85 (1.01)	0.77
Awake time (h)	5.25 (2.29)	5.57 (3.42)	4.10 (2.44)	0.22
Impedance (kΩ)	NA	5.01 (1.09)	NA	NA
No nicotine doses ( <i>n</i> )	14	13	9	NA
No coffee ( <i>n</i> )	11	12	9	NA

Values are reported as the mean (SD), unless otherwise indicated. Frequencies for participants who did not consume nicotine and caffeine 2 h before the experiment are displayed. NA, Not applicable.

\**p* Value for one-way ANOVA for variable × group interaction.

asures (RM; T1 pretest; T2 during stimulation, T3 post-test).

### Randomization and blinding

The participants were randomized into the following three groups: active tDCS group, 20 participants; sham tDCS group, 20 participants; control group, 20 participants). Every third participant was randomized to the control group by order of inclusion. The DC stimulator was set up for study mode, and was started by entering a 5 digit code in the display. Allocation to the active or sham tDCS group was performed by assigning each participant to an individual 5 digit code from a list consisting of 40 codes, where 20 were associated with active tDCS and 20 were associated with sham tDCS. The order of the treatment codes was randomized using a random number generator (randomize.org). The key to the individual treatment codes was kept separate from the experimenters, and thus the active and sham conditions were double blind.

### tDCS

tDCS was administered using a neuroConn DC Stimulator, a battery-driven device that constantly monitors electrical impedance and terminates the stimulation if the voltage exceeds safety limits. The stimulation duration was 20 min with an intensity of 2 mA. DC was transferred by a pair of 35 cm<sup>2</sup> (0.057 mA/cm<sup>2</sup>) rubber electrodes inserted into sponge pads soaked with 10 ml of medical grade sterile water. Electrode sponges were changed to a clean pair between participants. To reduce the skin sensation and achieve improved connection on the electrode-scalp interface, Ten20 neurodiagnostic electrode paste (Weaver and Company) was applied to the scalp at the site of stimulation. The electrode positioning was similar to that in the study by Fregni et al. (2006). In order to stimulate M1, the anode was placed at the C3 or C4 positions in the 10/20 system for EEG electrode positions, on the position contralateral to the dominant hand. The cathode was placed in the supraorbital area, contralateral to the anode. To reduce discomfort, the stimulation had fade-in and fade-out periods of 20 s. Sham tDCS consisted of an 8 s fade-in, followed by 30 s of DC stimula-

tion, and was terminated by a 5 s fade-out. The sham condition mimicked the skin sensation of active tDCS, but had insufficient duration to induce aftereffects in cortical excitability (Nitsche and Paulus, 2000).

### Outcomes

#### Grooved pegboard test

The grooved pegboard test (Lafayette Instrument) was used to test fine motor speed, visuomotor speed, and eye–hand coordination. The test consists of a board with 25 keyholes that requires keys to be correctly rotated and inserted. The keys are located on a tray above the keyholes. The participants were instructed to complete the test as fast as possible, and the outcome was evaluated in seconds from the start to all 25 keys correctly placed. The GPT was administered for the dominant hand (GPD) and for the nondominant hand (GPN) at all three time points.

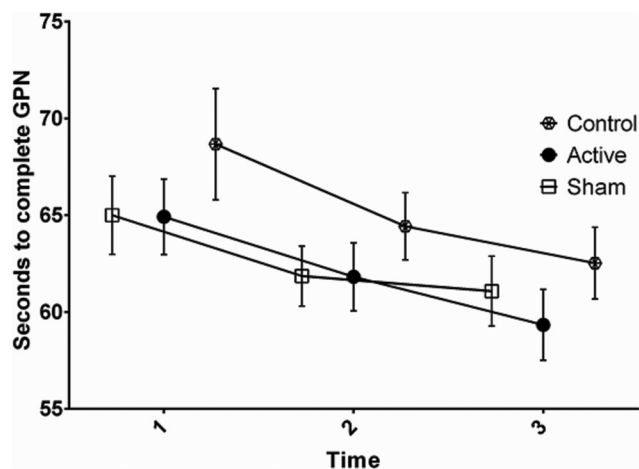
#### Trail making test

In order to test whether there were changes in dual and divided attention abilities, attention shift, and psychomotor speed, the TMT B (Halstead–Reitan Neuropsychological Battery) was used. The test consists of drawing a line between numbered points on a paper in the correct sequence. The participants were instructed to complete the test as fast as possible, and the outcome was evaluated in seconds from start to completion.

#### Individual differences

Before starting the study, the head circumference of the participants was measured. The BMI of the participants was calculated by measuring their weight and height. Hand dominance was registered by participant self-report. Sleep status was registered as the number of hours of sleep for the participant on the night before undergoing the test, and the number of hours awake at the time of testing. Caffeine and nicotine (cigarettes or chewing tobacco) use was registered as the number of doses in the 2 h preceding the study. Electric impedance (in kilo-ohm) was registered by reading the value in the stimulator display after 1 min of stimulation. In the sham condition, a random impedance value was displayed, and thus sham and active stimulation appeared similar to the experimenters.





**Figure 3.** Seconds to complete GPN at pretest (1), during stimulation (2), and post-test (3). Error bars denote the SEM.

were used to test the normal distribution of the variables. For the RM ANOVA, the effect sizes were calculated as partial  $\eta^2$  ( $\eta^2_p$ ). For the multiple linear regression effect, sizes were calculated as  $R^2$ . Mauchly's test was used to test the assumption of sphericity between the conditions. Alpha levels were set to  $\leq 0.05$ .

## Results

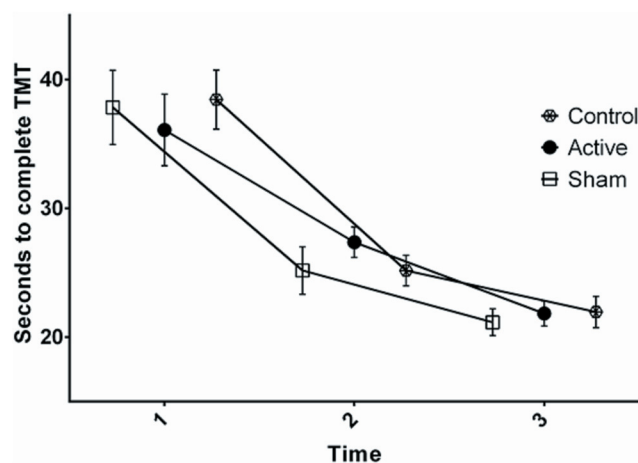
Prior to analyzing the effect of tDCS on the outcome measures, we compared the pretest results among the three groups using one-way ANOVA to eliminate the possibility that the differences observed after tDCS were due to the groups being different at baseline. One-way ANOVA indicated that the three groups had similar performances on the GPD ( $F_{(2,59)} = 0.07, p = 0.93^a$ ), GPN ( $F_{(2,59)} = 0.93, p = 0.40^b$ ) and TMT ( $F_{(2,59)} = 0.21, p = 0.81^c$ ) in the pretest. The data on GPD, GPN, and TMT were normally distributed (see Table 5, for definition of superscript designations).

### Effects of tDCS on GPT

On the GPD test, there was a significant effect of time ( $F_{(2,114)} = 39.76, p < 0.01, \eta^2_p = 0.41^d$ ), but no significant group  $\times$  time interaction term was observed ( $F_{(4,114)} = 0.18, p = 0.95, \eta^2_p = 0.01^e$ ). On the GPN test, there was a significant effect of time ( $F_{(2,114)} = 20.04, p < 0.01, \eta^2_p = 0.26^f$ ), but no significant group  $\times$  time interaction term was observed ( $F_{(4,114)} = 0.42, p = 0.79, \eta^2_p = 0.41^g$ ). The results indicated that while there was a general reduction in the number of seconds to needed to complete the GPD and GPN from pretest to post-test, there were no between-group differences.

### Effects of tDCS on TMT

For the TMT (Fig. 4), the degrees of freedom were corrected using Greenhouse–Geisser estimates of sphericity ( $\epsilon = 0.71$ ). The results show that the number of seconds needed to complete the test was reduced over time ( $F_{(2,114)} = 101.55, p < 0.01, \eta^2_p = 0.64^h$ ), but no significant group  $\times$  time interaction term ( $F_{(4,114)} = 0.79, p = 0.50, \eta^2_p = 0.03^i$ ) was observed.



**Figure 4.** Seconds to complete TMT at pretest (1), during stimulation (2), and post-test (3). Error bars denote the SEM.

### Individual differences

On the  $\Delta$  scores (T1 – T3), one-way ANOVA indicated there were no significant differences among the groups on the GPD ( $F_{(2,57)} = 0.27, p = 0.76^j$ ), GPN ( $F_{(2,57)} = 0.52, p = 0.59^k$ ), and TMT ( $F_{(2,57)} = 0.26, p = 0.77^l$ ). Furthermore, there were no differences between sham and control group T1 – T3 scores on the GPD ( $F_{(1,39)} = 0.44, p = 0.51^m$ ), GPN ( $F_{(1,39)} = 0.82, p = 0.37^n$ ), and TMT ( $F_{(1,39)} = 0.01, p = 0.94^o$ ), indicating no significant placebo effect. Because of the large observed unsystematic variance caused by naturally occurring individual differences in practice effect, compared to the variances that were a systematic effect of the experimental manipulation in the ANOVA, we conducted an exploratory partial correlation analysis with gender as a control variable to investigate the influence of individual differences on the T1 – T3 scores for the GPD, GPN, and TMT<sup>p</sup>. There was a positive correlation between BMI and TMT T1 – T3 ( $r = 0.28$ , two-tailed  $p = 0.03$ ). Since the impedance variable only was viable for the active tDCS group, a similar analysis was conducted, filtering out the Sham and Control groups, and included impedance<sup>q</sup>. In the active group, there were correlations between GPD T1 – T3 and the number of caffeine doses the last 2 h ( $r = -0.56$ , two-tailed  $p = 0.01$ ), and impedance ( $r = -0.64$ , two-tailed  $p < 0.01$ ). For GPN T1 – T3, no significant correlations with the individual difference variables were identified.

To further investigate the influence of variance in the significantly correlated ( $p < 0.05$ ) control variables on the T1 – T3 scores, we conducted two linear regression analyses: regression 1 with TMT T1 – T3 as the dependent variable, and BMI, gender, and dummy-coded group affiliation for the sham and control groups as predictors<sup>r</sup>; and regression 2 for the active group with GPD T1 – T3 as the dependent variable, and gender, number of caffeine doses the last 2 h before the test, and impedance as predictors<sup>s</sup> (Tables 2, 3).

The results from regression 1 indicate that BMI, but not group affiliation and gender, was a predictor for an increased practice effect on the TMT. The results from

**Table 2: Mean values for GPT with dominant and non-dominant hand (GPD / GPN) and TMT with confidence intervals on 1 pre-test, 2 during stimulation and 3 post-test, and T1-T3  $\Delta$  scores with 95% confidence intervals**

	Sham group		Active group		Control group	
	Mean	95% CI	Mean	95% CI	Mean	95% CI
GPD1	59.79	55.82–63.76	60.43	55.55–65.32	59.41	56.07–62.74
GPD2	55.12	51.69–58.55	55.61	52.20–58.72	55.26	52.38–58.14
GPD3	54.36	51.39–57.34	54.68	51.25–58.11	54.92	52.00–57.84
GPN1	65.00	60.78–69.21	64.62	60.54–68.70	68.67	62.67–74.68
GPN2	61.86	58.60–65.11	61.82	58.15–65.49	64.43	60.79–68.08
GPN3	61.08	57.30–64.85	59.34	55.51–63.18	62.53	58.65–66.41
TMT1	37.84	31.84–43.87	36.09	30.30–41.88	38.45	33.66–43.24
TMT2	25.18	21.33–29.04	27.37	24.89–29.85	25.17	22.69–27.65
TMT3	21.60	19.41–23.79	21.84	19.79–23.88	21.96	19.42–24.51
GPD $\Delta$	5.42	3.11–7.73	5.76	2.31–9.20	4.49	2.63–6.35
GPN $\Delta$	3.92	0.66–7.18	5.28	2.95–7.61	6.14	2.18–10.11
TMT $\Delta$	16.24	11.34–21.14	14.26	8.90–19.61	16.49	11.65–21.33

regression 2 indicate that, for participants who had received active tDCS, caffeine intake prior to participating in the study and electrode impedance were predictors for increased practice effects on the GPD. There was also a tendency toward male participants having an increased practice effect, but this did not reach significance.

### Adverse effects

In general, the stimulation was well tolerated by the participants, and no sessions were aborted due to adverse effects. The occurrence of adverse effects in the active and sham groups, and the mean difference in intensity between groups are displayed in table 4 (Table 4). The sensation of burning under the electrodes was significantly more intense in the active group, compared with the sham group, with a similar tendency for skin redness. In the active group, an exploratory correlation analysis was conducted to investigate the relation between impedance and the registered adverse effects. No correlations were significant ( $p < 0.05$ ).

### Discussion

The present study tested the effect of anodal 2 mA tDCS over the M1 on performance and practice effects in two commonly used neuropsychological tests, while controlling for the influence of interindividual differences in anatomy and functional state of the brain.

**Table 3: Statistics for regressions 1 and 2**

	<i>B</i>	<i>t</i>	$\beta$	<i>p</i>	Partial correlation
Regression 1					
Included variables					
BMI	0.77	2.26	0.29	0.03*	
Excluded variables					
Sham	0.04	0.30		0.76	0.04
Control	0.02	0.14		0.89	0.02
Gender	-0.15	-1.23		0.22	-0.16
Regression 2					
Included variables					
Gender	3.28	2.01	0.27	0.52	
Impedance	-1.77	-2.46	-0.32	0.02*	
Caffeine intake	2.11	0.75	0.37	0.01**	

### Effect of tDCS on performance and practice effect on GPT and TMT

The results indicated no effect of a 20 min session of anodal 2 mA tDCS over the M1 on performance and practice effects on the GPD and GPN, and on the TMT, compared with the sham and control groups. The active, sham, and control groups had equal performance before, during, and after stimulation. Furthermore, no placebo effect represented as the difference between the sham and the control groups was observed.

A consensus article stated that there are no aftereffects of tDCS found on GPT results (Ziemann et al., 2008). In the present study, it was hypothesized that the increased intensity of stimulation would produce stimulation effects that surpassed those of earlier studies. However, there were no effects of 2 mA 20 min anodal tDCS on performance or practice effect. An increased retention of a practice effect, which recently was demonstrated on older subjects after 1 mA stimulation (Parikh and Cole, 2014), cannot be ruled out, as the retention of a practice effect was not measured in the present study. The TMT is a commonly used neuropsychologic test that is sensitive for organic brain injury (Reitan, 1958) and can also serve as a measure for general psychomotor speed in healthy individuals. In the present study, the anodal stimulation of M1 had no effect on the performance and practice effect of this test. The results are similar to the findings in a recent study (Park et al., 2014) that applied bilateral frontal anodal tDCS (two stimulators) with extraencephalic cathodes on older subjects, and that also found no improvement on the TMT. However, in this study, the stimulation paired with computer-assisted training did improve verbal working memory. Consequently, it is possible that the TMT practice effect cannot be effectively improved by anodal tDCS, neither frontally nor over the motor cortex. In chronic stroke patients, anodal tDCS over the ipsilesional M1 has been shown to improve performance on specific motor tasks (Fregni et al., 2005; Hummel et al., 2005; Boggio et al., 2007). However, a more recent study (Rossi et al., 2013) on acute stroke patients failed to demonstrate effects on clinically relevant recovery. It is likely that the excitatory effects of anodal tDCS may produce different behavioral outcomes in

**Table 4: Frequency of sessions after which specific adverse effect occurred, and the mean intensity for specific adverse effects across all sessions defined as 0 = none, 1 = mild, 2 = moderate, 3 = severe.**

	Frequency by sessions		Mean intensity (SD)			<i>p</i> *
	Sham group ( <i>n</i> = 20)	Active group ( <i>n</i> = 20)	Sham group	Active group	Sham group	
Headache	6	2	0.60 (.94)	0.20 (.62)	0.120	0.120
Neck pain	2	0	0.20 (.62)	0	0.154	0.154
Scalp pain	3	3	0.40 (.99)	0.40 (1.05)	1	1
Tingling	8	8	0.80 (1.01)	0.80 (1.01)	1	1
Itching	5	6	0.55 (1.00)	0.75 (1.25)	0.580	0.580
Burning sensation	6	13	0.7 (1.13)	1.45 (1.19)	0.048**	0.048**
Skin redness	11	17	1.25 (1.25)	1.95 (.94)	0.053	0.053
Sleepiness	9	6	1.00 (1.21)	0.85 (1.42)	0.722	0.722
Trouble concentrating	1	3	0.10 (.45)	0.40 (.99)	0.226	0.226
Acute mood change	0	0	0	0	1	1
Others	1	1	.10 (.45)	.30 (.73)	0.304	0.304

\**p*-value for independent samples t-test for group differences in adverse effect intensity.

\*\**p* < .05.

healthy and impaired subjects. The participants in the present study were university students and had high test performances at pretest on the GPT (Table 2) compared with the normative group with  $\geq 13$  years of education (Ruff and Parker, 1993). If a ceiling effect on the test were present, it may have left limited room for improvement, and thus reduced the observed practice effect.

#### Effect of control variables on practice effects in GPT and TMT

For the entire sample, the results indicated that BMI was a weak predictor for practice effect on TMT where subjects with higher BMI achieving a slightly larger reduction in time to complete the test from pretest to post-test. As

gender was controlled for in the analysis, further attempts to explain this novel finding would be difficult without going outside the scope of this report. More interesting with regard to the hypothesis, when analyzing only participants who had undergone active tDCS, there were two significant predictors for increased practice effect in GPD, as follows: lower impedance values and higher caffeine intake prior to the study. However, these effects were not present in the GPN and TMT, and thus cannot be regarded as general effects. There was a tendency toward males achieving a larger practice effect, but this did not reach significance.

In the present study, lower electrode impedance predicted an increased practice effect on the GPD. The

**Table 5: Statistics**

		Data structure	Type of test	Power (%)
a	GPD	Normally distributed	One-way ANOVA	6
b	GPN	Normally distributed	One-way ANOVA	20
c	TMT	Normally distributed	One-way ANOVA	8
d	GPD RM time	Normally distributed	RM ANOVA	100
e	GPD*group	Normally distributed	RM ANOVA	9
f	GPN RM time	Normally distributed	RM ANOVA	100
g	GPN*group	Normally distributed	RM ANOVA	13
h	TMT RM time	Normally distributed	RM ANOVA	100
i	TMT*group	Normally distributed	RM ANOVA	9
j	$\Delta$ GPD	Normally distributed	One-way ANOVA	9
k	$\Delta$ GPN	Normally distributed	One-way ANOVA	13
l	$\Delta$ TMT	Normally distributed	One-way ANOVA	15
m	$\Delta$ GPD (sham and control)	Normally distributed	One-way ANOVA	81
n	$\Delta$ GPN (sham and control)	Normally distributed	One-way ANOVA	99
o	$\Delta$ TMT (sham and control)	Normally distributed	One-way ANOVA	12
p	Corr all	Non-normally distributed	Partial Pearson correlation, two-tailed	65
q	Corr active	Non-normally distributed	Partial Pearson correlation, two-tailed	25
r	Regression all	Non-normally distributed*	Linear multiple regression	97
s	Regression active	Non-normally distributed*	Linear multiple regression	41

Lines refer to the alphabetical value provided in the Results section. *Post hoc* power calculations were performed on the sampled data in G\*Power for Windows. For one-way and RM ANOVA, the *n* and SDs from the data with desired alpha level of 0.05 were used. For the correlation analysis, a hypothetical regression value of 0.03 against a zero correlation were used. For regression analysis, the observed  $R^2$  values were used. Deviations from normal distribution in the sample containing all participants were significantly non-normal: head circumference,  $D(60) = 0.15$ ,  $p < 0.01$ ; age,  $D(60) = 0.22$ ,  $p < 0.01$ ; doses of nicotine in last 2 h before undergoing stimulation,  $D(60) = 0.33$ ,  $p < 0.01$ ; doses of caffeine in last 2 h before undergoing stimulation,  $D(60) = 0.29$ ,  $p < 0.01$ ; hours awake,  $D(60) = .$ ,  $p = 0.02$ ; and hours of sleep,  $D(60) = 0.15$ ,  $p < 0.01$ . Deviations from normal distribution in the sample containing participants from the active group were significantly non-normal: age,  $D(20) = 0.21$ ,  $p = 0.02$ ; doses of nicotine in last 2 h before undergoing stimulation,  $D(20) = 0.40$ ,  $p < 0.01$ ; doses of caffeine,  $D(20) = 0.32$ ,  $p < 0.10$ ; and hours of sleep,  $D(20) = 0.21$ ,  $p = 0.02$ .

\*The assumptions for regression regarding homoscedasticity, independent errors and normally distributed errors were met.

predictive value of impedance on individual treatment outcomes have, to our knowledge, not been reported previously, and impedance levels are rarely reported or discussed in the literature. In tDCS, the target current intensity (in milliamperes) is set according to the stimulation protocol, and is thus a static value. The interelectrode impedance (in kilo-ohms) is the resistance in the flow path of electrons between the anode and the cathode, and is subject to individual differences. In the present study, a stimulator with automatic current control was used that automatically adjusted the voltage as per Ohm's law. Thus, participants with higher interelectrode impedance required increased voltage in order to drive the current. It has been demonstrated that high impedance values, and consequently increased voltage, lead to an increased risk of skin lesions after consecutive sessions of tDCS (Palm et al., 2008; Frank et al., 2010). The voltage has been shown to be a determinant for discomfort under the electrodes during stimulation, with a threshold of  $\sim 10$  V (Lang et al., 2005). In addition to discomfort, skin sensation under the electrodes can reduce the efficacy of patient blinding. This has been reported to be the case at stimulation intensities of 2 mA (O'Connell et al., 2012). As per Ohm's law, the voltage required to drive the current through a given impedance increases with increased intensity, and thus the risk of exceeding the threshold of 10 V. However, interelectrode impedance in human subjects is both time and current dependent, with peak values during the fade-in phase, with a decrease after the fade-in phase and incremental reduction throughout the stimulation period (Hahn et al., 2013). Furthermore, the factors that influence skin sensation under the electrodes are complex and not linearly or solely determined by voltage (Dundas et al., 2007). The present study used a current of 2 mA, and observed a mean impedance of 5.01 k $\Omega$ , resulting in a mean voltage of 10.02 V. The fade-in phase was 20 s, and the impedance levels were registered after 60 s, a time at which impedance levels were relatively stable. An exploratory correlation analysis indicated no significant correlation between impedance and the intensity of registered adverse effects, including tingling, scalp pain, and burning sensation under electrodes, indicating that the voltage did not exceed the threshold for discomfort. However, the registration form that was used in this study was designed for clinical adverse effects and might not be sensitive for discomforts at the lower end of the spectrum.

The results from the present study indicated that lower impedance predicted higher practice effect on the GPD. These results are difficult to interpret, as, to our knowledge, the relationship between electrode impedance and behavioral outcomes has not previously been demonstrated. Sulcus-gyro morphology in the human brain is complex and subject to large interindividual variations (Mangin et al., 2004), and is likely to affect the distribution of electric current in the brain during tDCS (Datta et al., 2012) and possibly also the electric resistance between the electrodes in tDCS. Thus, the observed individual differences in impedance in the present study may have correlated with individual differences in skull and brain

anatomy that were relevant to the effect of stimulation on the practice effect in the GPD.

In the present study, neither tDCS nor caffeine intake predicted an increased practice effect in the GPD on the sample as a whole, but increased caffeine intake 2 h before the study predicted an increased practice effect in the active tDCS group, but not in the sham and control groups. Caffeine has an effect on a range of behavioral outcomes such as alertness, reduced fatigue, and performance on simple tasks (Smith, 2002), but not on the GPT specifically (Lieberman et al., 1987). It is possible that, in the present study, the cumulative effect of tDCS induced excitability increase, and caffeine induced increase in alertness, might have exceeded the threshold required to facilitate the practice effect.

The effect of gender on excitability changes after tDCS have previously been described in the literature. Regarding changes in motor cortex neuroplasticity following tDCS, women had more immediate and prolonged inhibition following cathodal stimulation (Kuo et al., 2006), but there were no gender differences in excitability following anodal stimulation. Comparatively, regarding neuroplasticity in the visual cortex, no effect of cathodal stimulation was found, but a gender-specific effect of anodal stimulation occurred with immediate and prolonged facilitation in female participants, and a prolonged inhibitory effect in male participants (Chaieb et al., 2008). Considering these studies, the gender-specific effects of tDCS appear to be site specific. Regarding behavioral outcomes on motor performance and practice effect after tDCS, the results from the present study indicated a tendency toward a larger practice effect on the GPD for males compared to females in the active tDCS group. This tendency was not observed in the sham and control groups, and not on the GPN and TMT.

### Limitations

The stimulation in this study was delivered using two sponge electrodes, arranged in the relatively common M1-SO montage. Evidence from MRI-derived computer head models has indicated that the distribution of the electric field in the brain using this method is not focal (Mendonca et al., 2011); therefore, the hypothesis with which we applied anodal tDCS to the M1 might have been insufficiently precise with regard to the stimulation pattern. A high-definition tDCS technique would have made the distribution of electric field less widespread. However, the superiority of this method in terms of behavioral outcomes has not yet been demonstrated (Kuo et al., 2013). Therefore, it was considered interesting to test the effect of the relatively easy to apply M1-SO montage on neuropsychological tests with predictive value for real-life functional outcomes. The interpretation of the results may have been further complicated by the fact that the cathode was placed on the supraorbital area contralateral to the anode. It is therefore likely that the frontal cortex was under the influence of cathodal tDCS, which is known to produce inhibitory effects (Nitsche and Paulus, 2001). Furthermore, as the post-tests were performed immediately after the stimulation ended, the present study did not



investigate the aftereffects or long-lasting effects of the stimulation. Future studies could investigate the prolonged effects of tDCS on motor performance as a recent study (Parikh and Cole, 2014) observed an increased retention of practice effect on the GPT after stimulation with a lower intensity than that used in the present study. Furthermore, the GPT and TMT were administered in the same order in every trial and in every group. Therefore, the order of the tests might have systematically affected the outcomes. Nicotine use in the present study was registered as “doses of nicotine” (any type of tobacco for oral intake), and thus the habitual consumption pattern, or whether the participants were under the influence of withdrawal effects, was not controlled for. Finally, the participant’s habitual pattern of caffeine consumption was not controlled for in the analyses. High and low habitual caffeine consumers may have responded differently to both intake and abstinence (Rogers et al., 2013), and thus the effect of caffeine intake 2 h before the experiment on the outcomes should be interpreted with caution.

## Conclusion

Contrary to the hypothesis, the stimulation had no effect on performance and practice effect on GPT and TMT. However, uniquely for the participants who received active tDCS, caffeine intake in the last 2 h before the study and lower electrode impedance predicted a larger practice effect for the GPD. Based on the current study, it is recommended that future studies that use tDCS on human subjects report electrode impedance, in addition to stimulation parameters and the method of electrode preparation. This may, as the present results suggests, affect the outcomes of the stimulation.

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