No effect of anodal tDCS on motor cortical excitability and no evidence for responders in a large double-blind placebo-controlled trial

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ABSTRACT

Background: Transcranial direct current stimulation (tDCS) has emerged as a non-invasive brain stimulation technique. Most studies show that anodal tDCS increases cortical excitability. However, this effect has been found to be highly variable.
Objective: To test the effect of anodal tDCS on cortical excitability and the interaction effect of two participant-specific factors that may explain individual differences in sensitivity to anodal tDCS: the Brain Derived Neurotrophic Factor Val66Met polymorphism (BDNF genotype) and the latency difference between anterior-posterior and lateromedial TMS pulses (APLM latency).
Methods: In 62 healthy participants, cortical excitability over the left motor cortex was measured before and after anodal tDCS at 2 mA for 20 min in a pre-registered, double-blind, randomized, placebo-controlled trial with repeated measures.
Results: We did not find a main effect of anodal tDCS, nor an interaction effect of the participant-specific predictors. Moreover, further analyses did not provide evidence for the existence of responders and non-responders.
Conclusion: This study indicates that anodal tDCS at 2 mA for 20 min may not reliably affect cortical excitability.

Introduction

Transcranial direct current stimulation (tDCS) has emerged as a non-invasive brain stimulation technique with potential applications in a variety of clinical fields, ranging from rehabilitation medicine [1] to psychiatry [2]. Research on tDCS increased rapidly with over 900 PubMed publications on tDCS in 2018 compared to 7 in 2001. On a physiological level, previous studies have shown that anodal tDCS causes *long-term potentiation* (LTP) [3], which is modulated by γ-*aminobutyric acid* (GABA) [4] and mediated by the *N-methyl-D-aspartate* (NMDA) receptor [5], the α-*amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid* (AMPA) receptor [6] and *Brain Derived Neurotrophic Factor* (BDNF) [7]. Most studies show that anodal tDCS increases cortical excitability [8,9]. However, this effect has been found to be highly variable [10,11]. Furthermore, a recent study in human cadavers indicated that tDCS may need to be applied at very high intensities to induce a meaningful effect on neuronal spiking and subthreshold currents [12].

Two specific factors have been suggested to explain individual differences in sensitivity to tDCS: the Brain Derived Neurotrophic Factor Val66Met polymorphism (BDNF genotype) and the latency difference between anterior-posterior and lateromedial TMS pulses (APLM latency). BDNF is known to be involved in long-term potentiation and is thought to mediate the effect of tDCS on cortical excitability [13,14] and motor learning [7]. The Val66Met polymorphism of this gene occurs in roughly a third of the Caucasian population [15,16] and decreases the amount of BDNF that is released from activated cells. Previous studies have reported

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that carriers of this polymorphism show more pronounced increases in cortical excitability after anodal tDCS compared to non-carriers [13,14].

The APLM latency is a measure of indirect wave recruitment in the motor cortex [17]. *Transcranial magnetic stimulation* (TMS) pulses that induce a current in the anterior-posterior (AP) direction are thought to recruit indirect waves from projections of premotor areas, whereas TMS pulses that induce a current in the lateral-medial (LM) direction are thought to recruit direct waves directly from pyramidal cells in layer V of the primary motor cortex [18]. The difference in the onset latency of the *motor-evoked potentials* (MEP) between the two coil directions is called the AP-LM latency difference [11,17]. In this study, we abbreviate this as the APLM latency. Shorter APLM latencies are thought to reflect more efficient early indirect wave recruitment. Since early indirect wave inputs are thought to target regions on or close to the pyramidal cell body and tDCS is thought to depolarize the pyramidal cell body [19], the efficiency of early indirect wave recruitment may predict the effect of tDCS. Indeed, previous studies have reported that individuals exhibiting shorter APLM latencies show increased cortical excitability after anodal tDCS, whereas individuals exhibiting longer APLM latencies may not [11,20].

If these participant-specific predictors have an interaction effect with tDCS in healthy individuals, they could be used to establish potential responders among patients for later clinical applications. However, an important limitation of the studies that investigated the interaction effects of BDNF genotype and APLM latency is the absence of a placebo (sham) tDCS condition [11,13,14,20]. This limits both the estimation of the real effect size of anodal tDCS and the estimation of an empirical threshold for responders and non-responders. Furthermore, since the interaction effects of BDNF genotype and APLM latency were measured in separate populations, it cannot be excluded that these predictors are correlated and that one apparent predictor is merely reflecting the predictive value of the other actual predictor.

Therefore, the primary goal of this study is to investigate the main effect of anodal tDCS on cortical excitability and the interaction effects of BDNF genotype and APLM latency on this main effect in a pre-registered, double-blind, randomized, placebo-controlled trial with repeated measures and sufficient power. Participants in our study were tested in both sham and anodal tDCS conditions and their BDNF genotype, as well as APLM latency, were obtained. We hypothesized 1) that anodal tDCS would increase the amplitude of *motor evoked potentials* (MEPs); 2) that the BDNF Val66Met polymorphism would be associated with a stronger effect of anodal tDCS; 3) that a shorter APLM latency would be associated with a stronger effect of anodal tDCS. Furthermore, we explored whether the effect of tDCS was stronger when measured with higher TMS intensities.

Materials and methods

Subjects

Sixty-two participants (21 men and 41 women; mean age: 24.6, range [19 48]) were included in this study. All participants were right-handed according to the Oldfield handedness questionnaire [21]. Participants did not display any contraindications to tDCS or TMS stimulation, as tested by the TMS adult safety questionnaire [22], nor did they take any medication influencing the central nervous system or had a history of psychiatric or neurological disease. All participants gave written consent and were instructed not to consume any caffeinated drinks 2 h prior to the experiment. Smokers included in the study were not restricted in their nicotine intake [23]. The study was approved by the Medical Ethical Committee of the Erasmus MC Rotterdam and performed following the Declaration of Helsinki. Furthermore, this study was pre-registered in the open science framework [24].

Experimental procedure

We used a double-blind, randomized, placebo-controlled design with repeated measures. Participants were tested four times, with the first two and last two sessions constituting measurement pairs. In each measurement pair, a participant received one anodal and one sham tDCS stimulation, resulting in a total of two anodal and two sham sessions per participant. The order of the sessions within measurement pairs was randomized and counterbalanced. During the experiment, participants were seated in a comfortable chair with their right hand resting pronated on a table. Before and after tDCS stimulation, their cortical excitability was measured by applying TMS pulses over the left motor cortex while measuring MEPs in the *electromyography* (EMG) signal over the right *first dorsal interosseous* (FDI) muscle. At the end of each session, participants filled in a questionnaire in which they could indicate if they thought they received real (anodal) or placebo (sham) tDCS on a 5-point Likert-Scale (1 = 'Certainly Placebo'; 2 = 'Probably Placebo'; 3 = 'I don't know'; 4 = 'Probably tDCS'; 5 = 'Certainly tDCS'). Additionally, participants were asked verbally whether they experienced any side effects during the current session or after any previous sessions. Sessions were scheduled 48 h apart to avoid carry-over effects in cortical excitability [25,26]. The experimental procedure was identical in all sessions, except for 40 additionally-applied TMS pulses in the first two sessions to measure the APLM latency. At the end of the last session, a saliva sample was taken to determine BDNF genotype.

TMS: setup

Monophasic single-pulse TMS was applied using a Visor2 XT system (ANT Neuro, The Netherlands), consisting of a MagPro X100 stimulator, a MC-B70 coil (MagVenture, Denmark), a custom-built amplifier (TMSi, The Netherlands), a Polaris Spectra motion tracking system (NDI, Canada), and Visor2 software (ANT Neuro, The Netherlands). The EMG signal was acquired from the right first dorsal interosseous muscle with silver-silver chloride electrodes using a belly-tendon montage. The raw signal was sampled at 5 kHz and stored on a computer for offline analysis. Before pulses were applied, the head of a participant was co-registered to a stock MRI scan with at least 100 data points over the scalp and reference points of the nasion and pre-auricular points. Next, the hotspot, defined as the location where the largest MEPs could be detected, was determined using a pseudorandom motor mapping protocol at a fixed stimulation intensity [27]. Throughout the experiment, visual feedback of the current and previous coil positions was shown on a monitor and MEP amplitudes at these positions were color-coded. At the hotspot, the *resting motor threshold* (RMT), defined as the lowest stimulation intensity that has $\geq$ 50% chance to elicit an EMG response with a peak-to-peak amplitude of 50 μV, was determined with the Motor Threshold Assessment Tool (MTAT 2.0) [28]. The RMT in the posterior-anterior current direction (PA; coil held 45$°$ from the midsagittal line with the handle pointing caudal and laterally) was established in every session. Additionally, we measured the RMT in anterior-posterior (AP; 180$°$ to the PA-position) and lateromedial (LM; with the handle pointing 90$°$ leftwards from the midsagittal line) current direction in the first two sessions to establish the APLM latency.

TMS: APLM latency

In the first two sessions, we measured the APLM latency by applying 10 TMS pulses in AP and 10 TMS pulses in the LM current direction. APLM latency measurements were conducted two times in the first session and two times in the second session. TMS pulses were applied while participants pinched a force meter at 10% of *maximal voluntary contraction* (MVC). The *active motor thresholds* (AMTs) for each coil direction were calculated based on the corresponding RMT (90% of the RMT). AP pulses were applied at 110% AMT to elicit indirect waves and LM pulses were applied at 150% AMT, or at least 50% of the maximum stimulator output, to ensure the recruitment of direct waves [17].

TMS: cortical excitability

To measure cortical excitability, TMS pulses were applied in the PA current direction over the previously determined hotspot while participants rested their hand on a table. TMS pulses were separated by 3−4 s [29]. Cortical excitability was assessed before tDCS (Baseline A and Baseline B) and at three time-points after tDCS (T0A: immediately after stimulation, T15B: 15 min after stimulation, and T30A: 30 min after stimulation). We used two measurement protocols to establish cortical excitability: A) applying 30 pulses at 120% of RMT followed by 30 pulses at 160% of RMT and B) establishing an Input-Output Curve consisting of 30 pulses at 120% of RMT, 30 pulses at 160% of RMT, and 10 pulses at each of the following stimulation intensities: 80%, 90%, 100%, 110%, 130%, 140%, 150%, 170%, 180% of RMT in a pseudo-random order. At the time-points Baseline A, T0A and T30A we used protocol A and at time-points Baseline B and T15B we used protocol B (Supplementary Figure 1). This yielded 30 pulses at 120% of RMT and 30 pulses at 160% of RMT at each time-point, resulting in a total of 60 pre-tDCS and 90 post-tDCS pulses for both of these intensities.

Transcranial direct current stimulation

Anodal transcranial direct current was applied over the left motor cortex using a DC-stimulator (NeuroConn, Germany; Model-No: 0008). Saline-soaked 5×7cm sponge electrodes were used and a 2 mA current (current density 0.057 mA/cm2) was applied for 20 min [14,20] while keeping the hand in a relaxed position. The cathode was placed over the right supra-orbital area with the longer side of the electrode placed along the transverse plane and the center of the anode was placed over the previously marked hotspot with the longer side of the electrode placed along the coronal plane. In the anodal tDCS condition, the current was ramped up for 45 s, held constant at 2 mA for 20 min, and ramped down again for 45 s. During stimulation, impedance was monitored continuously. In the sham condition, the current was ramped up for 45 s to 2 mA and ramped down to 0 mA for 45 s, after 40 s of stimulation, producing skin sensations that mimic anodal stimulation. To ensure double-blinding, the double-blind mode of the DC-stimulator was used. In the double-blind mode, a code entered in the machine determines whether sham or anodal tDCS is applied. A matrix with four columns of codes was created by another researcher before the first participant was included. Rows corresponded to participants (order of inclusion) and columns correspond to measurement sessions.

BDNF genotype

In the last measurement session of each participant, saliva samples were collected in Oragene-DNA tubes (DNA Genotek, Canada). BDNF Val66Met polymorphism was genotyped with Taqman Allelic Discrimination using the Assay-On-Demand service of Life Technologies as described before [30]. Participants carrying at least one Met allele were classified as "met-carriers" and participants carrying two Val alleles were classified as "non-carriers" [14].

Data analysis

Offline data analysis was conducted using custom-made MAT-LAB scripts (Mathworks, USA). The EMG traces were filtered with a 6th-order high-pass Butterworth filter with a cutoff frequency of 1 Hz. Subsequently, EMG traces with TMS artifacts were automatically reconstructed by fitting and subtracting a two-term exponential curve. The fit of these reconstructions was checked by visual inspection.

The APLM latency was calculated by first rectifying and then averaging the 10 AP traces and the 10 LM traces. In each averaged trace, the onset threshold was defined at 20 standard deviations above the average pre-response period (5−15 ms after TMS pulse). The APLM onset latency was calculated by subtracting the onset latency in the LM current direction from the onset latency in the AP direction. For each participant, we used the median of the four APLM latency measurements as a participant-specific predictor in the statistical analysis.

Cortical excitability was calculated in two steps. First, EMG traces were excluded from analysis if the root mean square of the background EMG, 100-5 ms before the TMS pulse, was larger than 10 μV. Of the remaining trials, we calculated the MEP amplitude, defined as the peak-to-peak amplitude between 5 ms and 60 ms after TMS stimulation. Cortical excitability at 120% of RMT was used in the primary analysis. Cortical excitability at 160% of RMT was used in the exploratory analysis to investigate whether the effect of tDCS was stronger when measured at higher TMS intensities. Cortical excitability at TMS intensities other than 120% and 160% of RMT was only used to visualize the Input-Output Curve.

Primary analysis

In the primary analysis, we investigated cortical excitability with a TMS intensity of 120% RMT. For each session of each participant, we used the ratio between the grand average MEP amplitude after tDCS (90 pulses divided over three time points) and the grand average MEP amplitude before tDCS (60 pulses at baseline) as the dependent variable in the statistical analysis.

In the statistical analysis, we estimated the main effect of tDCS on cortical excitability and the interaction effects of the participant-specific predictors: APLM latency and BDNF genotype. We used a linear mixed-model to accommodate the nested design of the study (two anodal tDCS and two sham tDCS sessions per participant) [31]. Furthermore, this allowed us to estimate the between-participant and the within-participant variability. We used five versions of a linear mixed model: a (1) Basic Model, (2) BDNF Model, (3) APLM Model, (4) Full Model, (5) Null Model.

$$MEP_{p,s} \sim \alpha_p + \beta 1_p * TDCS_{p,s} + \varepsilon \tag{1}$$

$$MEP_{p,s} \sim \alpha_p + \beta 1_p*TDCS_{p,s} + \beta 2*BDNF_p + \beta 3*BDNF_p*TDCS_{p,s} + \varepsilon \tag{2}$$

$$MEP_{p,s} \sim \alpha_p + \beta 1_p*TDCS_{p,s} + \beta 2*APLM_p + \beta 3*APLM_p*TDCS_{p,s} + \varepsilon \tag{3}$$

$$MEP_{p,s} \sim \alpha_p + \beta 1_p*TDCS_{p,s} + \beta 2*BDNF_p + \beta 3*BDNF_p*TDCS_{p,s} \\ + \beta 4*APLM_p + \beta 5*APLM_p*TDCS_{p,s} + \varepsilon \tag{4}$$

$$MEP_{p,s} \sim \alpha_p + \varepsilon \tag{5}$$

For each session s of participant p, $MEP_{p,s}$ is the MEP ratio -1 and $TDCS_{p,s}$ indicates whether tDCS was anodal ($TDCS_{p,s} = 1$) or sham ($TDCS_{p,s} = 0$). Furthermore, $BDNF_p$ indicates whether the participant is a met-carrier ($BDNF_p = 1$) or a non-carrier ($BDNF_p = 0$) and $APLM_p$ is defined as the participant-specific APLM latency in milliseconds. To prevent a shift in the estimations when comparing different versions of the model, we subtracted the group average from the participant-specific predictors ($BDNF_p$ and $APLM_p$).

Individual differences in the intercept (α_p), and the main effect of tDCS ($\beta 1_p$) were modeled as random effects. The group average intercept, the group average main effect of tDCS and all other effects were modeled as fixed effects. Estimations were considered significant when the 95% confidence interval of an estimate did not include zero. Finally, we compared how well each version of the model explained the data by comparing the *Akaike Information Criterion* (AIC) and the *Bayesian Information Criterion* (BIC) values for each model.

We used an a priori power calculation with data simulations to determine the number of participants needed in this experiment. The details of the a priori power analysis can be found in the pre-registration [24]. In short, we expected the main effect of tDCS to be 0.35 [11], the interaction of BDNF genotype to be 0.30 [14], and the interaction effect of APLM latency to be -0.23/ms [11]. These are unstandardized effect sizes (difference in MEP ratio or MEP ratio per millisecond). Furthermore, we expected BDNF genotype and APLM latency to be correlated (rho = 0.25). Because the interaction effect of BDNF genotype required the most participants, we powered the number of participants on this interaction effect in the BDNF model. In the pre-registration phase, we aimed for 80 participants. However, as a result of a low enrollment rate of volunteers in the recruitment phase, we decided to settle for 62 participants, since this amount of participants provided sufficient a priori power ($\geq 90\%$) for the main effect of tDCS, the interaction effect of BDNF genotype and the interaction effect of APLM latency (Supplementary Figure 2).

Exploratory analysis

After the primary analysis, we performed 6 exploratory analyses. First, we performed post-hoc power calculations for Models 1−3. Second, we performed the primary analysis again, including only the participants of which the average baseline MEP amplitude was within the two middle quartiles (N = 29). This was done to see whether the between-participant variability in average baseline MEP amplitude across participants influenced the results of the primary analysis in a meaningful way. Third, to assess whether reduced inter-session intervals affected our results, we repeated the primary analysis, including only the participants of which all

inter-session intervals were at least 48 h (N = 44). Fourth, we assessed whether the main effect of tDCS was larger when cortical excitability was measured with higher TMS intensities. To do so, we used Model 1 with the MEP ratios at 160% RMT. Furthermore, we plotted the Input-Output Curves to visualize cortical excitability at the stimulation intensities surrounding 120% and 160% of RMT. Fifth, we used a model similar to Model 2 and 3 to assess whether there was an interaction effect between the participant-specific RMT and the effect of anodal tDCS [32,33].

Finally, we performed a responder analysis consisting of 3 steps. In step 1, we calculated the average MEP ratio of the two sham sessions and the average MEP ratio of the two anodal tDCS sessions for each participant. We used the 'fitgmdist.m' function in Matlab to check if the averages of the participants (anodal tDCS and sham) were best explained by one, two or three clusters. In step 2, we calculated the *smallest detectable change* (SDC) between two sham sessions. In step 3, we plotted the differences between the anodal and sham session (net effect) in the first measurement pair against the differences in the second measurement pair and calculated the Pearson correlation.

Results

There were five participants with missing data: One participant dropped out after one measurement pair, due to pain on the scalp during the TMS pulses. No other side effects were reported. Furthermore, genotyping failed in three participants. Finally, after deblinding and reassessing the stimulation codes, we found that one of the participants received 3 sham sessions and 1 anodal session. This participant, as well as the participant that dropped out after the first measurement pair, were excluded from the responder analysis (N = 60), as two measurement pairs are needed for this analysis. In the primary analysis and all other analyses, we removed the three participants with missing genetics but included the other two participants. In the primary analysis, 1% of the *electromyography* (EMG) traces were reconstructed due to TMS artifacts and 4% of the trials were removed due to muscle contractions in the baseline period. In all participants, TMS stimulation at 160% of the *resting motor threshold* (RMT) was below 100% of maximum stimulator output (MSO). The average inter-session interval was 138.5 h (std = 110.2 h). In 15 sessions (out of 234) the inter-session interval was slightly below 48 h (mean = 45 h, range [40 47.5]).

Taken together, the linear mixed model contained 234 sessions (116 anodal and 118 sham) of 59 participants. There were 34 non-carriers and 25 met-carriers, including 1 homozygous met-carrier. The mean APLM latency (mean = 3.7 ms, range = [1.2 6.2]) and the RMTs were stable over sessions (Supplementary Figure 3). BDNF genotype and APLM latency were not correlated (rho = 0.05, $p = 0.717$). Regarding the blinding for the conditions, participants guessed the stimulation condition correctly in 50%, incorrectly in 17%, and chose '*I don't know*' in 33% of the time in all sessions combined. Correct guesses increased and choosing the '*I don't know*' option decreased over measurement sessions (Supplementary Figure 4).

Primary analysis

Table 1 shows the group average RMT, the baseline MEP at 120% of RMT and the baseline MEP at 160% of RMT in all sessions. Table 2 shows the estimates of the different linear mixed models. None of the models showed a main effect of tDCS on cortical excitability. Furthermore, the BDNF Model did not show an interaction effect of the BDNF genotype and the APLM Model did not show an interaction effect of APLM latency. The cortical excitability in all sessions was best explained by the Null Model (Model 5), which only

Table 1
Descriptive statistics (average ± standard deviation) of the *resting motor threshold* (RMT) and the amplitude of the *motor evoked potentials* (MEPs) at baseline (N = 59). The bottom row shows the estimated baseline differences between the anodal and the sham conditions, as calculated with a mixed linear model similar to Model 1. Results show no difference between RMTs or MEP baselines.

	RMT (%MSO)	120% of RMT Baseline MEP (µV)	160% of RMT Baseline MEP (µV)
All Sessions	**43 ± 6**	**1735 ± 1623**	**3721 ± 2660**
First Sham Session	43 ± 7	1636 ± 1748	3628 ± 2915
First tDCS Session	44 ± 7	1808 ± 2159	3791 ± 3295
Second Sham Session	43 ± 7	1764 ± 1587	3744 ± 2648
Second tDCS Session	43 ± 7	1759 ± 1578	3797 ± 2354
TDCS	0.3	81	101
[95% CI]	[-0.3 1.0]	[-137 300]	[-176 378]
p value	*0.279*	*0.464*	*0.474*

includes the participant-specific intercepts but no tDCS, BNDF, and APLM effects. In this Null Model, the within-subject standard deviation was 0.33 (CI 95% [0.30 0.37]), and the between-subject standard deviation was 0.18 (CI 95% [0.13 0.26]). The intercept was 0.14 (CI 95% [0.08 0.21]), which indicates that in all sessions (sham and anodal tDCS), cortical excitability was approximately 14% higher in the post-stimulation time-points (average of T0A, T15B, and T30A) compared to baseline.

Figure 1 illustrates the results of the primary analysis. Evolution of cortical excitability over the different time-points was similar in the sham and anodal tDCS sessions (Figure 1 A–B) with no differences between carriers of the BDNF polymorphism compared to non-carriers (Figure 1 C–D). Also the effect of tDCS was not related to participants' APLM latency (Figure 1 E–F).

Exploratory analysis

In the post-hoc power calculation, we used the data of the 59 participants in the primary analysis (BDNF genotype, APLM latency, number of anodal sessions, number of sham sessions) and the estimated within-subject and between-subject variance of the Null

Model to simulate datasets with different effect sizes. The post-hoc power calculation indicated that this study had sufficient power (≥90%) to find the expected effects (Supplementary Figure 5).

We found that between-participant variability in the baseline MEP did not influence the results of the primary analysis in a meaningful way. Including only the participants of which the average baseline MEP amplitude at 120% of RMT was within the two middle quartiles, stongly reduced the between-participant variability (baseline MEP averaged over all sessions = 1329 ± 356 µV). However, when including only this subset of participants, cortical excitability was still best explained by the Null Model (Supplementary Table 1).

Furthermore, we found that the reduced inter session intervals did not influence the results of the primary analysis. We repeated the primary analysis including only the participants of which all inter-session intervals were at least 48 h (N = 44). Again, none of the models showed a main effect of tDCS on cortical excitability (Supplementary Table 2).

In addition to the interaction effects of BDNF genotype and APLM latency, we did not find evidence for an interaction effect between the participant-specific RMT and tDCS (Beta = <0.01; df = 230; p = 0.574; CI 95% = [-0.01 0.02]; Supplementary Figure 6). Furthermore, we did not find a main effect of tDCS when cortical excitability was measured at 160% of participants' RMT (Beta = −0.02; df = 232; p = 0.647; CI 95% = [-0.09 0.05]; Supplementary Figure 7A and 7B).

The results of the responder analysis are shown in Figure 2. Average responses were better explained by one single cluster rather than two clusters (Figure 2 B–C). The fitgmdist.m function could not converge using three clusters. The SDC between two sham sessions (0.91) was similar to the SDC between two anodal tDCS sessions (0.90) (Figure 2 D-E). In Figure 2 A, providing an overview of responses in all measurements of all participants, it is illustrated how the SDC between two sham sessions can be used as a response limit to determine the chance that an individual participant has responded to tDCS in a measurement pair. Lastly, the difference between anodal and sham tDCS in the first measurement pair was not related to the difference between anodal and sham tDCS in the second measurement pair (Figure 2 F).

Table 2
Results of the primary analysis (N = 59). Each column corresponds to a different version of the linear mixed model.

| Variables	(1) Basic Model MEP Ratio	(2) BDNF Model MEP Ratio	(3) APLM Model MEP Ratio	(4) Full Model MEP Ratio	(5) Null Model MEP Ratio
Intercept[p]	0.16	0.16	0.16	0.16	0.14
[95% CI]	[0.09 0.24]	[0.09 0.23]	[0.09 0.24]	[0.09 0.23]	[0.08 0.21]
p value	*p < 0.001*	*p < 0.001*	*p < 0.001*	*p < 0.001*	*p < 0.001*
TDCS	-0.04	-0.04	-0.04	-0.04	
[95% CI]	[-0.13 0.06]	[-0.13 0.06]	[-0.13 0.06]	[-0.13 0.05]	
p value	*0.429*	*0.429*	*0.427*	*0.427*	
BDNF		-0.08		-0.08	
[95% CI]		[-0.22 0.07]		[-0.22 0.07]	
p value		*0.285*		*0.293*	
TDCS*BDNF		0.09		0.09	
[95% CI]		[-0.10 0.28]		[-0.09 0.28]	
p value		*0.361*		*0.330*	
APLM			-0.02	-0.02	
[95% CI]			[-0.10 0.06]	[-0.09 0.06]	
p value			*0.646*	*0.686*	
TDCS*APLM			-0.05	-0.05	
[95% CI]			[-0.15 0.05]	[-0.15 0.05]	
p value			*0.318*	*0.293*	
Fit statistics					
(AIC)	198.24	200.88	200.11	202.72	199.25
(BIC)	215.52	225.07	224.30	233.82	209.62
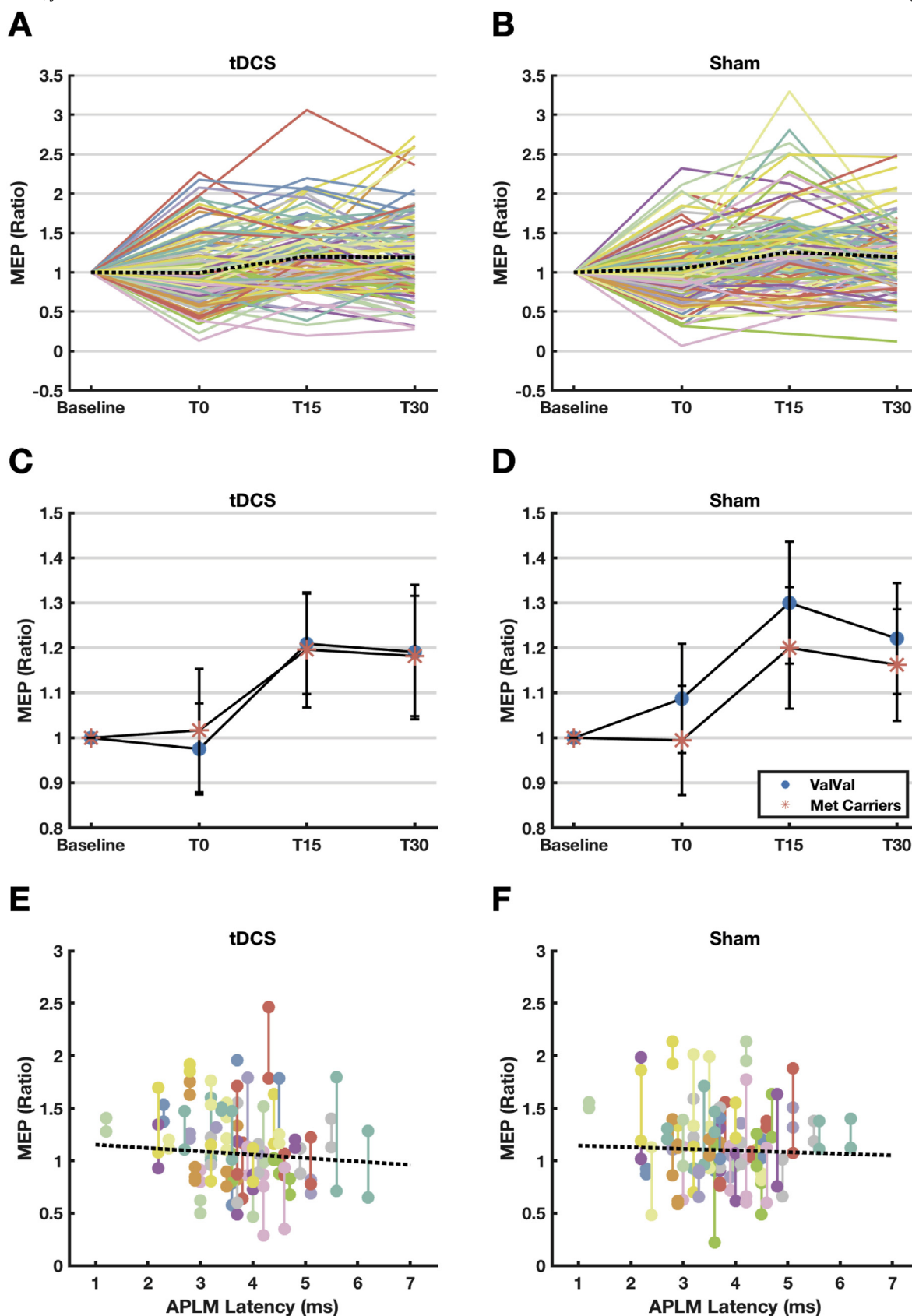
Fig. 1. Effects on cortical excitability (N = 59). **A-B**: Individual (colored lines) and mean (black dotted line) responses to anodal tDCS and sham stimulation over time (T1: 0 min after stimulation, T15: 15 min after stimulation, and T30: 30 min after stimulation). The evolution of cortical excitability is similar between tDCS and sham stimulation over different time points. **C-D**: Responses of carriers (red asterisks) and non-carriers (blue dot) of the BDNF polymorphism to anodal tDCS or sham stimulation. Error bars indicate the 95% CI of the mean. The evolution is similar between carriers and non-carriers **E-F**: Relation between APLM latency and mean MEP ratio after anodal tDCS or sham stimulation. Connected dots illustrate responses of the same individual in two anodal tDCS and two sham conditions respectively. The black dotted line represents the regression line. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
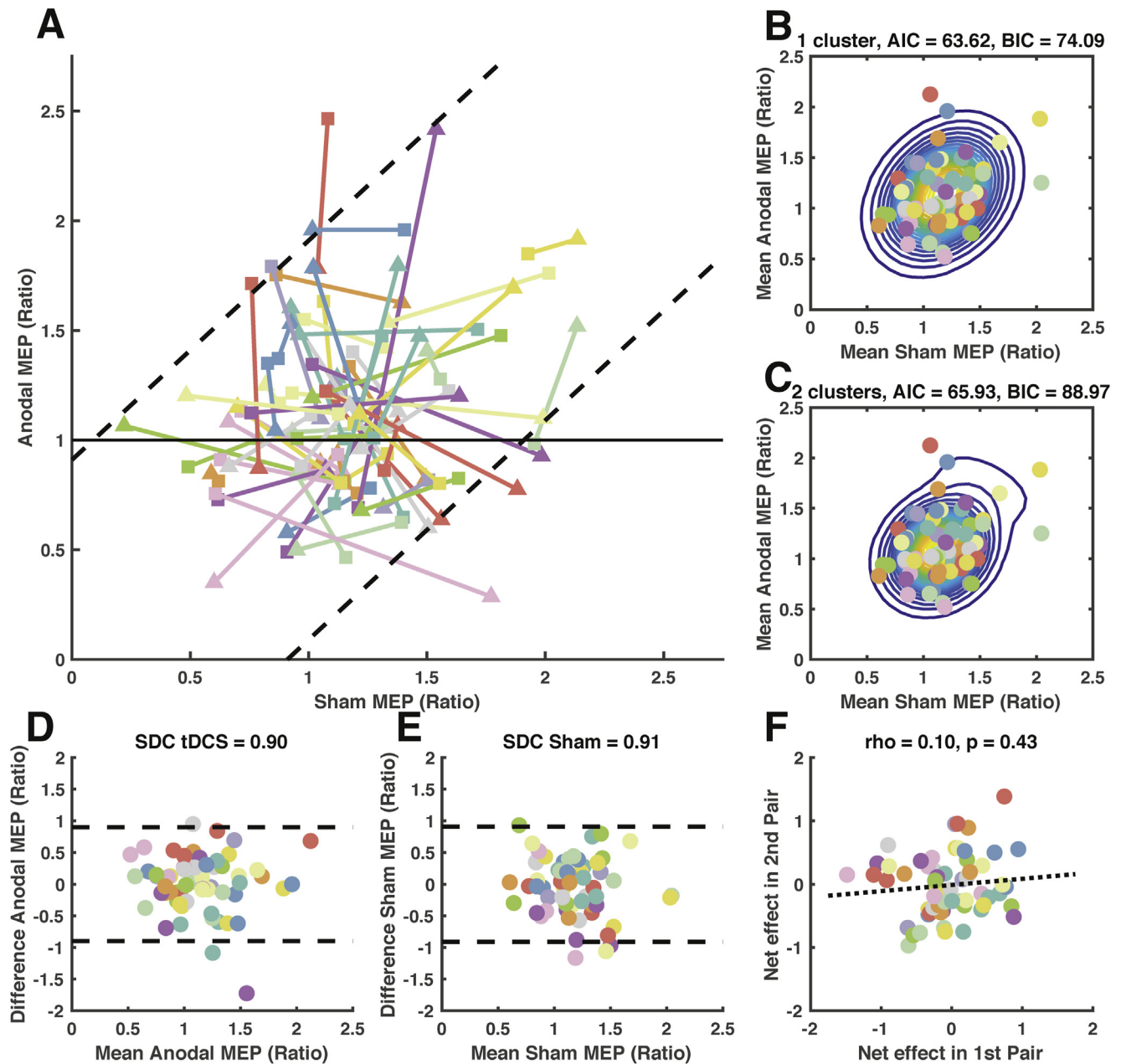
Fig. 2. Results of the responder analysis (N = 60). **A:** Overview of individual responses to anodal tDCS and sham stimulation in both measurement pairs. A measurement pair consists of one anodal tDCS session and one sham session. Triangles illustrate responses in the first, squares in the second measurement pair. The horizontal line depicts an arbitrary response threshold in studies without a control condition. Dashed lines depict our proposed threshold for measurement pairs based on the *smallest detectable change* (SDC) in sham sessions (see D-E). **B–C:** Mean response to anodal tDCS stimulation plotted against mean response to sham stimulation. Cluster analysis shows that the distribution of average responses is better explained by a single cluster than by two clusters. The colored dots represent the participants and the colored lines represent the fitted distribution at 20 equally spaced steps. The model fit is shown above the panels. **D-E:** Bland-Altman plots depicting the *smallest detectable change* (SDC) between two anodal tDCS sessions and two sham sessions. **F:** Net effects in both measurement pairs. The net effect is the difference in the MEP ratio between the anodal tDCS session and the sham session. The net effect in a first measurement pair was not related to the net effect in a second measurement pair.

Discussion

In this pre-registered, double-blind, randomized, placebo-controlled trial with repeated measures, anodal tDCS at 2 mA for 20 min did not affect cortical excitability on a group level, despite sufficient post-hoc power to find even small differences in MEP ratio between anodal and sham tDCS. Moreover, we did not find an interaction effect between anodal tDCS and two suggested participant-specific predictors: BDNF genotype and APLM latency. Finally, we did not find an interaction effect between the

participant-specific RMT and the main effect of tDCS. This corroborates recent null findings [34,35] with high-quality evidence. However, it is important to note that many previous studies did find an effect of anodal tDCS on cortical excitability [8,9], including a recent study with a similar stimulation protocol [36].

A recent modelling study indicated that TMS pulses that induce anterior-posterior (AP) and posterior-anterior (PA) currents target spatially segregated populations in the crown and lip of the precentral gyrus [37], where in most individuals the caudal part of the dorsal premotor cortex is located [38]. While this modelling study

challenges the current interpretation of the APLM latency [11,17,18], it may provide an alternative explanation for the variability in the effect of tDCS between individuals and experiments. New developments, such as advanced tDCS protocols that use magnetic resonance imaging and high-definition tDCS to preferentially target the premotor cortex of individuals may, therefore, improve the reliability of anodal tDCS [39].

Responders and non-responders

In a post-hoc analysis, we found no evidence for the existence of subgroups of responders and non-responders to anodal tDCS. In this analysis, a positive response was defined as an anodal tDCS session in which cortical excitability increased significantly compared to sham, and a responder was defined as an individual who has consistent positive responses in both anodal tDCS sessions. Most previous studies did not test for subpopulations and defined responders via arbitrary thresholds [35,40,41]. Furthermore, the previously reported cluster analyses that did test for subpopulations used only a single tDCS session to define responders [10,11]. In this study, we included both the sham and anodal tDCS sessions in a mixed model cluster analysis and did not find evidence for a subpopulation of participants that are more sensitive to anodal than to sham tDCS (Figure 2 B–C). Moreover, the net effect of tDCS in the first measurement pair was not related to the net effect in the second measurement pair, which suggests an absence of responders altogether (Figure 2 F).

For future studies, we propose that a positive response should be determined based on a measurement pair consisting of one real and one sham tDCS session and that the *smallest detectable change* (SDC) between two sham sessions should be used as a threshold. Figure 2 A illustrates that if we would have used an arbitrary threshold of 1, all anodal and sham tDCS sessions above the horizontal line would be falsely classified as positive responses. Dashed lines in Figure 2 A depict how the SDC between two sham sessions can be used to define positive and opposite responses. In measurement pairs outside the dashed lines, there is less than a 5% chance that the anodal and sham session come from the same distribution. In light of these results, past studies may have falsely classified variability in the measurement as positive responses to tDCS, and subsequently used these constructed subgroups to explore participant specific predictors.

Strengths and limitations

Strengths of this study are the large sample size, the pre-registration of the study design and analysis, the double-blinding, the placebo (sham) control, and the use of frameless neuro-navigation [42]. The power and the pre-registration are the most critical strengths since the average power of tDCS publications was found to be below 50% [43], which increases the chance of finding false-negative and false-positive results and increases sensitivity to biases, such as selective analyses, selective reporting and selective publication [44].

An important limitation of this study comes from the small but significant increase in excitability in all sessions. An increase in excitability in the sham condition has been reported previously [34,45] and we hypothesize that this increase is caused by a cumulative effect of single-pulse TMS [46,47]. It has also been suggested that such increases in excitability might be due to changes in arousal or attention [34]. Although the increase in excitability seen in our study is smaller than the estimated effect size of anodal tDCS [9], we cannot exclude that it interfered with the effect of anodal tDCS in a non-linear fashion. Future studies should consider using a longer interpulse interval to prevent cumulative effects [46] and

restricting the number of TMS pulses to the ones necessary for the reliability of the primary analysis. Another option could be to capitalize on the cumulative effect of single-pulse TMS and investigate whether tDCS during the single-pulse TMS enhances the effect on cortical excitability [7]. Additionally, large, randomized studies which can reliably discern the effects of number and interval of single-pulse TMS will be needed to address cumulative effects.

The sham condition also creates potential limitations. Low currents are applied in the sham stimulation to blind participants by mimicking the sensation of real stimulation. However, previous findings show that stimulation durations of less than 3 min do not induce after-effects in cortical excitability [48]. It is therefore highly unlikely that 40 s of stimulation at 2 mA in the sham condition would have affected our null finding. Even in a hypothetical scenario, in which the sham condition of the double-blind mode would have affected cortical excitability, we would have expected to see duration-dependent differences (20 min versus 40 s of stimulation) between the conditions as reported by previous studies [3,36]. Furthermore, to ensure blinding of the experimenter, impedance is continuously checked and displayed. This is done with current pulses every 550 ms at a maximum of 200 μA, resulting in an average current of 6 μA after ramp down. It is highly unlikely that these low currents pass the skull and affect cortical excitability.

Additionally, participants may have potentially become aware of the sham stimulation. Despite the double-blinded sham condition, participants became progressively better at guessing the stimulation condition correctly over sessions. Yet, correct guesses increased to merely 57% in the last session and it is therefore unlikely that this influenced the results notably. Nonetheless, future studies with a within-subjects design could consider using a numbing cream to ensure similar levels of blinding over repeated measurements [49,50].

Finally, there are limitations due to the non-linear effects of tDCS found in previous studies, such as delayed effects [51], prolonged effects [52], and interaction effects in the intensity-duration parameter space [36]. Therefore, we cannot extrapolate the results of this study beyond 30 min after anodal tDCS stimulation, nor can we generalize the results of this study to other tDCS stimulation protocols.

Conclusion

To our knowledge, this is the largest study examining the effect of tDCS on cortical excitability thus far. We did not find a main effect of tDCS, nor did we see an interaction effect between tDCS and two participant-specific predictors for sensitivity to tDCS: BDNF genotype and APLM latency. Moreover, the additional analyses did not provide evidence for the existence of a subgroup of responders altogether. These results suggest that anodal tDCS at 2 mA for 20 min may not reliably affect cortical excitability. New developments, such as explorations of the intensity-duration parameter space [36] and network stimulation [53], may improve the reliability of tDCS.

CRediT authorship contribution statement

Zeb D. Jonker: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Carolin Gaiser:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing - original draft, Visualization. **Joke H.M. Tulen:** Writing - review & editing. **Gerard M. Ribbers:** Writing - review & editing. **Maarten A.**

Frens: Writing - review & editing, Supervision. **Ruud W. Selles:** Writing - review & editing, Supervision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2020.11.005.

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