tDCS Anodal tDCS increases bilateral corticospinal excitability irrespective of hemispheric dominance

Simin Rahman MS	Department of Neurology, Institute of Neurosciences, India
Ummatul Siddique MS	Department of Neurology, Institute of Neurosciences, India
Ashlyn Frazer Ph.D.	Department of Physiotherapy, Monash University, Australia
Alan Pearce Ph.D.	School of Allied Health, La Trobe University, Australia
Dawson Kidgell Ph.D.	Department of Physiotherapy, Monash University, Australia

Background: Transcranial direct current stimulation (tDCS) is a non-invasive brain stimulation technique that utilizes weak direct currents to induce polarity-dependent modulation of corticospinal excitability. Although tDCS exerts a modulatory effect over the stimulation region, several studies have also demonstrated that distal areas of the brain connected to the region of stimulation may also be affected, as well as the contralateral hemisphere. ***Objective:*** We examined the effect of a single session of anodal tDCS on corticospinal excitability and inhibition of both the stimulated and non-stimulated hemisphere and examined the influence of these responses by the brain-derived neurotrophic factor (BDNF) polymorphism. ***Methods:*** In a randomized cross-over design, changes in corticospinal excitability and inhibition of the stimulated and non-stimulated hemispheres were analysed in 13 participants in both the dominant and non-dominant primary motor cortex (M1). Participants were exposed to 20 min of anodal and sham tDCS and also undertook a blood sample for BDNF genotyping. ***Results:*** TMS revealed a bilateral increase in corticospinal excitability irrespective of which hemisphere (dominant vs non-dominant) was stimulated (all $P < 0.05$). Furthermore, the induction of corticospinal excitability was influenced by the *BDNF* polymorphism. ***Conclusion:*** This finding shows that anodal tDCS induces bilateral effects in corticospinal excitability irrespective of hemispheric dominance. This finding provides scientists and medical practitioners with a greater understanding as to how this technique may be used as a therapeutic tool for clinical populations.

Citation

Rahman S, Siddique U, Frazer A, Pearce A, Kidgell D. (2020). Anodal tDCS Increases Bilateral Corticospinal Excitability Irrespective of Hemispheric Dominance. Journal of Science and Medicine 2(2): 1-17.

Introduction

Altering the excitability of cortical neurons using electrical stimulation has been of particular interest for scientific and medical communities for over a decade [1]. Transcranial direct current stimulation (tDCS) has emerged as a popular non-invasive brain stimulation (NIBS) technique which involves the application of weak direct currents to the scalp. Transcranial magnetic stimulation (TMS) studies have revealed that tDCS over the primary motor cortex (M1) elicits temporary modifications in corticospinal excitability in a polarity-specific manner [2,3]. Anodal tDCS induces facilitatory effects on motor-evoked potentials (MEPs), while cathodal tDCS leads to inhibitory effects [3].

tDCS is believed to modulate the resting membrane potential of the pyramidal neurons in layer 5 [3-5] with a single session of tDCS (current intensities of 0.6 mA to 2 mA) applied for 5-20 mins increasing corticospinal excitability for up to 90 min post stimulation [3,6-9]. The after-effects of tDCS is well documented, with the general consensus attributing changes in synaptic strength due to the modulation of the N-Methyl-D-aspartate (NMDA) receptor [10]. Pharmacological studies have highlighted the importance of the NMDA receptor whereby the after-effects of anodal tDCS were suppressed when using the NMDA receptor antagonist dextromethorphane [11,12].

There has been extensive research examining the changes in M1 plasticity following anodal tDCS [3,6-9], whereby TMS was used to measure indices of M1 plasticity on the stimulated hemisphere. Interestingly, other NIBS techniques have shown to modulate not only the intended stimulated tissue but also distal connecting tissue and structures, as well as the opposite non-stimulated hemisphere [13]. Critically, emerging evidence from TMS studies show diverse findings regarding the direction of excitability of the non-stimulated hemisphere following various NIBS techniques [13-17]. Specifically, repetitive transcranial magnetic stimulation (rTMS) at 1 Hz and paired associative stimulation (PAS) has shown to increase excitability of both the stimulated and non-stimulated M1 [13,18,19] and decrease interhemispheric inhibition (IHI) between the left and right M1[9]. Similarly, Lang et al. [16] found that 10 min of anodal and cathodal tDCS at 1 mA modulated transcallosal inhibition. However, this finding was not accompanied by a bilateral increase in M1 excitability, with only an increase in MEP amplitude seen in the stimulated M1. In contrast, various protocols using intermittent theta bust stimulation (iTBS) have shown increases in corticospinal excitability of the stimulated hemisphere and a decrease in corticospinal excitability of the non-stimulated hemisphere [14,15,17]. Importantly, many of these studies used a dominant M1 arrangement whereby the stimulated hemisphere was the dominant M1 (left) and non-stimulated hemisphere was the non-dominant M1 (right). Previously, it has been shown that the non-dominant hemisphere has lower motor thresholds, higher MEPs [20] and shorter cortical silent period durations [21], suggesting a hemispheric difference in baseline characteristics. An interesting question to address is whether the magnitude of bilateral corticospinal plasticity is affected by the direction of stimulation (dominant vs non-dominant M1 stimulated), and, if there is a greater scope for the induction of corticospinal plasticity of the non-dominant hemisphere.

Interestingly, studies have reported that the modulation of corticospinal excitability and inhibition following NIBS techniques has been accompanied by improvements in motor performance [7,22,23] , in particular the cross-transfer of motor skills [24]. Recently, Hendy et al. [25] reported an increase in maximal strength and cross-activation to the contralateral untrained limb (left hand) following a single session of anodal tDCS applied to the ipsilateral right M1 during strength training of the right hand. Given that the cross-transfer of strength following training is thought to be due to an increase in excitability of the ipsilateral M1 [26], it would be apparent that the bilateral effects of anodal tDCS need to be clearly understood. Indeed, if anodal tDCS increases excitability in both the stimulated and non-stimulated hemispheres, this NIBS technique may be vital to further exploit the cross-transfer phenomenon in clinical settings. Conversely, if anodal tDCS decreases excitability of the non-stimulated hemisphere, it may counteract the cross-transfer effect by either reducing the capacity of the strength gained in the training arm or the adaptations within the ipsilateral M1.

It appears that individual corticospinal responses to tDCS are highly variable and the *BDNF polymorphism* has been identified as a potential contributing factor [27-29]. The influence of the *BDNF polymorphism* on the induction of M1 plasticity has also been observed following other NIBS techniques such as rTMS [28,30] and in older adults following anodal tDCS [29]. Critically, to our knowledge, there are no studies of whether the *BDNF polymorphism* influences the induction of corticospinal plasticity to the non-stimulated hemisphere, and, if the change in corticospinal excitability is proportional to the stimulated hemisphere. Therefore, the purpose of this study was to examine the effect of a single session of anodal tDCS on indices of corticospinal excitability and inhibition of both the stimulated and non-stimulated hemisphere. In particular, we examined corticospinal excitability/inhibition and the influence on these responses by the *BDNF*

polymorphism. We hypothesized that induction of experimentally-induced corticospinal plasticity (increased cortical excitability and reduced cortical inhibition) would be evident in both the stimulated and non-stimulated M1 regardless of which hemisphere was stimulated (dominant vs non-dominant), but the magnitude of these responses would be influenced by the *BDNF* polymorphism.

Materials and Methods

Participants

Thirteen participants (5 women, 8 men aged 18-35 years) volunteered to participate. All volunteers provided written informed consent prior to participation in the study, which was approved by the Human Research Ethics Committee in accordance with the standards by the Declaration of Helsinki. All participants were right-hand dominant as determined by the Edinburgh Handedness Inventory [31] with an LQ score of 86 ± 5 and were free from any known history of peripheral or neurological impairment. Prior to the experiment, all participants completed the adult safety screening questionnaire to determine their suitability for TMS and tDCS [32].

Experimental approach

Figure 1 outlines the organization of the study. After obtaining consent, participants completed a familiarization session 1 week prior to the study and were exposed to single-pulse TMS. In a double-blinded cross-over design, all participants were exposed to 20 min of anodal tDCS over the dominant (anode over the left M1; Figure 2i) and non-dominant (anode over right M1; Figure 2ii) M1, and, 20 min of sham tDCS (half the participants using the dominant M1 arrangement, the other half using the non-dominant M1 arrangement). The order of the conditions was counterbalanced and randomized between participants, with a wash-out period of 1 week between each condition [33]. Both tDCS conditions followed the identical testing protocol as shown in Figure 1 for the right and left Biceps Brachii (BB) muscles. Similarly, the order of muscle testing (right and left BB muscles) was counterbalanced and randomized between participants. All participants underwent TMS prior to and following the tDCS intervention. Participants were required to attend 3 separate sessions where they were exposed to 20 min of anodal (dominant M1 and non-dominant M1 arrangements) and sham tDCS applied at 2 mA with a current density of 0.08 mA/cm^2.

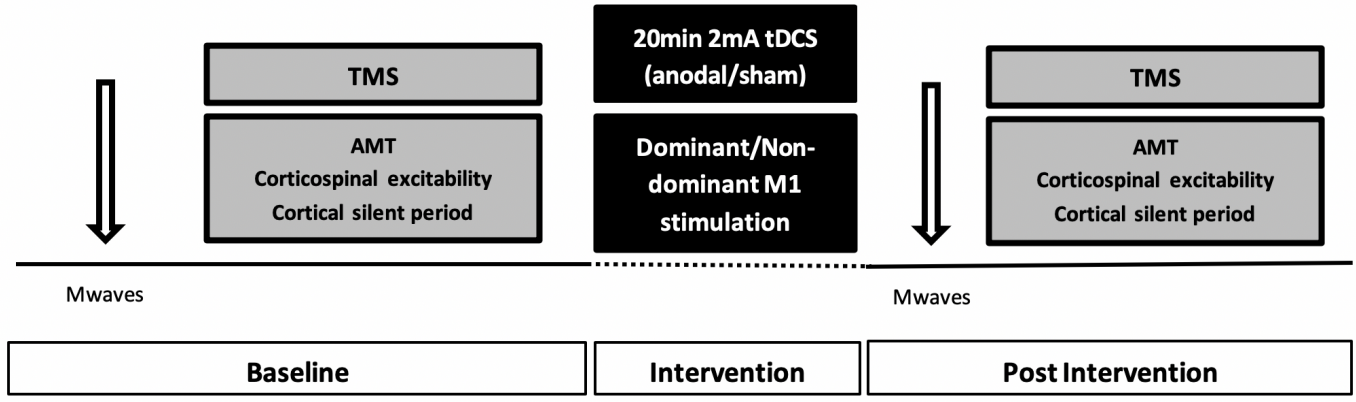

Figure 1. *Schematic representation of the experimental design with measures obtained prior and following sham and anodal tDCS (dominant and non-dominant M1 stimulation). Pre and post measures included assessment of peripheral muscle excitability (M-waves), corticospinal excitability and corticospinal inhibition of the stimulated and non-stimulated hemispheres.*

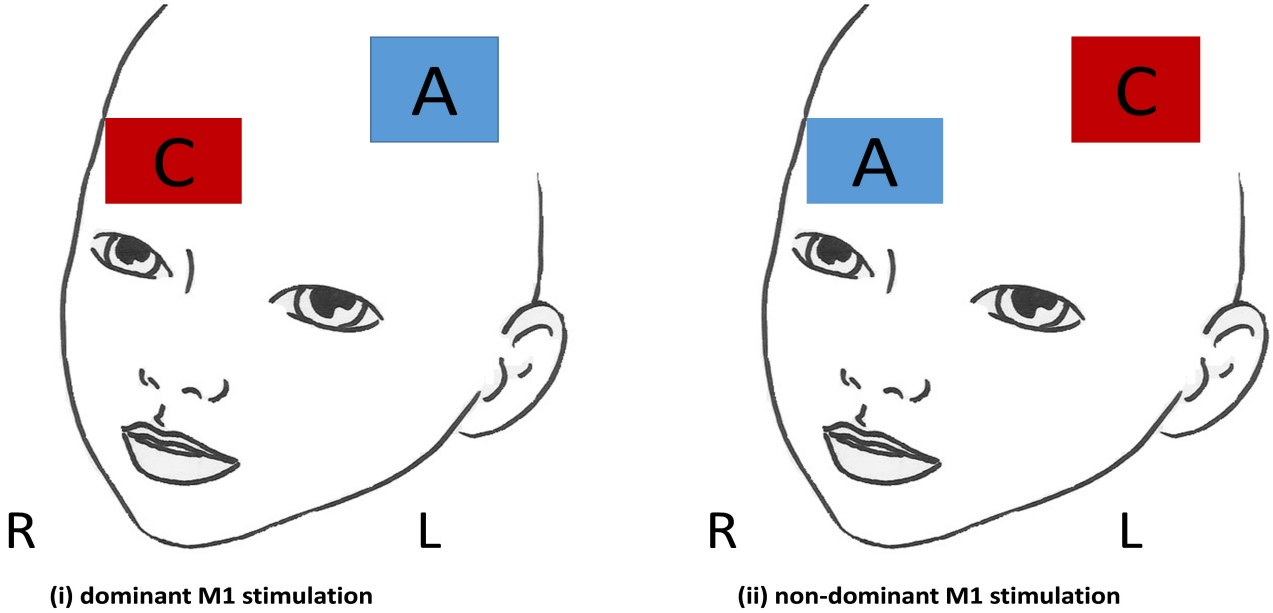

Figure 2. *Schematic representation of the two tDCS electrode arrangements used.* ***(i)*** *dominant M1 stimulation whereby the anode was fixed over the optimal cortical representation of the right BB muscle and the cathode was placed over the right contralateral supra orbital area* ***(ii)*** *non-dominant M1 stimulation whereby the anode was fixed over the optimal cortical representation of the left BB muscle and the cathode was placed over the left contralateral supra orbital area.*

Root mean square electromyography and maximal voluntary isometric contraction

To determine the maximal root mean square of the surface EMG (*rms*EMG) of both the right and left BB muscles, participants were seated in a chair, shoulders relaxed with their elbow flexed at 90 degrees. With the hand supinated and the force transducer (Futek Force Transducer LSB302, Melbourne) positioned over the middle aspect of the palmar surface of the hand, the participant was instructed to push up against the transducer as forcefully as possible for 3 sec. Three trials were performed; each trial was 3 sec in duration, separated by 3 min rest to minimize fatigue. The *rms*EMGduring maximal voluntary isometric contractions (MVIC) was calculated from a 500 ms segment occurring during the peak asymptote of MVIC force [34]. The greatest force output and corresponding surface EMG served as the MVIC and maximal*rms*EMG.

Surface electromyography

The area of electrode placement was shaven to remove fine hair, rubbed with an abrasive skin gel to remove dead skin, and then cleaned with 70% isopropyl alcohol. Surface electromyography (sEMG) was recorded from the right and left BB muscles using bipolar Ag-AgCl electrodes. The site of measurement was determined by marking the skin two thirds of the distance between the acromion and the lateral epicondyle, while the participant stood relaxed in the anatomical position [35]. This mark was then extended to the most anterior point of the muscle bulk, and as described by Wilson et al. [36] the electrodes were placed 2 cm apart over the mid-belly of the BB, with a ground electrode secured on the lateral epicondyle of the Humerus. sEMG signals were amplified (x1000), band pass filtered (high pass at 13 Hz, low pass at 1000 Hz), digitized online at 2 kHz, recorded (1 sec), and analyzed using Power Lab 4/35 (AD Instruments, Bella Vista, Australia).

Transcranial magnetic stimulation

TMS was delivered using a Magstim 200^2 stimulator (Magstim Co, Dyfed, UK) and a single figure-of-eight coil (external diameter of each loop 70 mm). The motor hotspot for the right and left BB muscles (with posterior-to anterior-induced current flow in the cortex) was determined, and active motor threshold (AMT) was established as the intensity at which at least 5 of 10 stimuli produced motor evoked potential (MEP) amplitudes of greater than 200 μV in the right and left BB muscles respectively. Following the tDCS intervention, AMT was retested and adjusted if required. To ensure all stimuli were delivered to the optimal motor hotspot throughout testing, participants wore a tight-fitting cap marked with a latitude-longitude matrix, positioned with reference to the nasion-inion and interaural lines.

During a low-level isometric contraction of the right and left BB muscles (4 ± 1% of maximal *rms*EMG), 10 single-pulse stimuli were delivered at 150% and 170% AMT. Participants were required to maintain an elbow joint angle of 90 degrees elbow flexion. Joint angle was measured with an electromagnetic goniometer (ADInstruments, Bella Vista, Australia), with visual feedback provided on a screen visible to both the participant and the researcher [37]. This joint position equated to 4 ± 1% of maximal *rms*EMG, with consistent muscle activation confirmed by recording pre-stimulus *rms*EMG for the 100-ms epoch before the delivery of each stimulus (Table 1).

		Sham tDCS		DH atDCS		N-DH atDCS		
		Pre	Post	Pre	Post	Pre	Post	P value
AMT SI (%)	Stimulated M1	41.54 ± 9.01	42.50 ± 9.37	44.31 ± 6.74	44.54 ± 6.67	41.46 ± 6.81	42.39 ± 6.59	0.28
	Non-Stimulated M1	45.08 ± 10.67	45.00 ± 10.60	41.77 ± 6.30	41.69 ± 6.23	44.00 ± 7.03	43.39 ± 7.35	0.98
Mwave (mV)	Stimulated M1	9.41 ± 4.72	9.53 ± 5.11	8.92 ± 2.84	8.96 ± 2.84	9.46 ± 3.35	9.42 ± 3.31	0.30
	Non-Stimulated M1	10.67 ± 6.05	10.81 ± 6.23	11.05 ± 5.15	11.13 ± 5.51	11.56 ± 5.80	11.59 ± 5.94	0.36
SP rmsEMG (% $MVIC_{MAX}$)	Stimulated M1	4.26 ± 2.12	4.65 ± 2.81	3.78 ± 2.27	4.48 ± 1.87	3.50 ± 2.16	3.69 ± 1.69	0.68
	Non-Stimulated M1	3.72 ± 1.69	3.53 ± 2.12	3.84 ± 2.12	3.76 ± 2.37	3.41 ± 1.55	3.26 ± 1.55	0.99

Table 1. *Mean (± SD) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus rmsEMG for the stimulated and non-stimulated M1 prior to and following sham and anodal tDCS (dominant and non-dominant M1 stimulation).* DH atDCS: anodal tDCS applied over the dominant M1; N-DH atDCS: anodal tDCS applied over the non-dominant M1; AMT SI: active motor threshold stimulus intensity. Single pulse (SP) *rms*EMG was pooled across both intensities (150% and 170% AMT). *P* values represent the 3 (conditions) x 2 (hemisphere) x 2 (time) repeated measures ANOVA used to determine any differences between conditions, hemispheres and time for the dependant variables AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG.

Maximum compound muscle action potential

Direct muscle responses were obtained from the right and left BB muscles by supramaximal electrical stimulation (pulse width, 200 μs) of the brachial plexus at Erbs point (DS7A; Digitimer, Hertfordshire, United Kingdom). The stimuli were delivered while the participant sat in an upright position, with the elbow at 90 degrees elbow flexion holding 4 ± 1% of maximal *rms*EMG. This low level of muscle activity was used to match the conditions under which TMS was delivered. An increase in current strength was applied to Erbs point until there was no further increase observed in the amplitude of the sEMG response (M_{MAX}). To ensure maximal responses, the current was increased an additional 20% and the average M_{MAX} was obtained from five stimuli, with a period of 6–9 s separating each stimulus. M_{MAX} was recorded at baseline and following the tDCS intervention

to control for possible changes in peripheral muscle excitability that could influence MEP amplitude.

Transcranial direct current stimulation

In all tDCS conditions, participants received 20 min of tDCS delivered by a battery-driven constant current transcranial direct current stimulator (NeuroConn, Ilmenau, Germany). Stimulation was delivered by a pair of conductive rubber electrodes (anode 25 cm^2; cathode 35 cm^2; current density 0.08 mA/cm^2) each soaked in saline solution (0.9% NaCl) and secured on the head with a rubber strap [2]. Anodal tDCS involved 20 min at 2 mA stimulation intensity, with a current density of 0.08 mA/cm^2. For the dominant M1 arrangement, the anode was fixed over the optimal cortical representation of the right BB muscle, as identified by TMS over the left cortex, and the cathode was placed over the right contralateral supra orbital area. For the non-dominant M1 arrangement, the anode was fixed over the optimal cortical representation of the left BB muscle, as identified by TMS over the right cortex, and the cathode was placed over the left contralateral supra orbital area (Figure 2i and ii). To ensure consistency of the site of stimulation, the participant's head was marked with a latitude-longitude matrix, positioned with reference to the nasion-inion and interaural lines. Both the experimenter and participant were blinded to the tDCS condition (i.e. sham versus anodal tDCS) through the use of codes on the tDCS machine. Using the protocol suggested by the international consensus paper on NIBS techniques [38], the sham protocol had the identical arrangement to the anodal tDCS condition, using both the dominant and non-dominant M1 arrangements (50% each), but the stimulation terminated after approximately 20 sec. This resulted in the participant experiencing the initial sensation of tDCS, however no experimental effects occurred. To obtain the participant's perception of discomfort throughout all tDCS conditions, discomfort (which included pain, itching, and tingling sensations) was assessed using a visual analogue scale (VAS) during the first 3 minutes of stimulation. The VAS ranged from 0 to 10 as visually described in cm units: 0 cm indicates "no discomfort" and 10 cm means "extremely uncomfortable".

BDNF Genotyping

As described by Frazer et al. [27], blood samples were obtained and participants were genotyped for the *BDNF Val66Met* polymorphism. Whole blood was obtained in EDTA tubes, and DNA was extracted using the QiaAmp DNA Mini Kit (Qiagen, N.V) according to the manufacturer's protocol. Briefly, 200 µl of whole blood was added to 20 µl of protease, followed by addition of 200 µl lysis buffer (Buffer AL). Samples were pulse-vortexed for 15 sec, briefly centrifuged (4000 rpm, 15 sec), then incubated at 56 °C for 10 min. Following incubation, 200 µl of absolute ethanol was added, the samples were again pulse-vortexed for 15 sec, and centrifuged (4000 rpm, 15 sec). The samples were then transferred to a QIAamp mini-column and centrifuged at 8,000 rpm for 1 min. The QIAamp mini-column was then placed in a clean 2 ml collection tube, and the used collection tube containing filtrate was discarded (this process was completed following each wash). Following this, 500 µl of wash buffer 1 (Buffer AW1) was added to the samples and centrifuged at 8000 rpm for 1 min. This process was repeated with wash buffer 2 on 2 occasions (Buffer AW2), and then the columns were transferred to a 2 ml collection tube and centrifuged at 14,000 rpm for 1 min to completely dry the membrane. To elute the DNA from the spin column, 150 µl of nuclease-free water (Life Technologies, Mulgrave, VIC) was added to the membrane and incubated at room temperature for 5 min, followed by centrifugation at 8,000 rpm for 1 min. The DNA concentration was determined using the NanoDrop 2000 (NanoDrop products, Wilmington, DE), and samples were diluted to 2.5 ng/µl and stored at -80 °C until further analysis [27].

The *Val66Met* single nucleotide polymorphism in the *BDNF* gene was typed by a polymerase chain reaction (PCR) in a total of 25 µl containing 125 ng of DNA, 10 x buffer (Life Technologies), 1.5 mM magnesium chloride ($MgCl_2$) (Sigma-Aldrich, St Louis, MO), 200 µM deoxyribonucleotide triphosphate (dNTP) (Life Technologies), 400 µM of each primer and 1 U Taq polymerase (Life

Technologies) using a thermal cycler (Takara Bio, Shiga, Japan). In accordance with Neves-Pereira et al. [39], primer sequences included ACTCTGGAGAGCGTGAATGG / AGAAGAGGAGGCTCCAAAGG. PCR started with an initial denaturation at 95°C for 5 min, followed by 94°C for 30 s, 60°C for 30 s, and 72°C for 30s for 30 cycles, with a final extension at 72°C for 5min. The PCR product was then digested with the restriction enzyme FastDigest PmlI (Eco72I) (Thermo Scientific, Massachusetts, USA). Briefly, 10 µl of the PCR sample was added to 17 µl of nuclease-free water (Life Technologies), 2 µl of 10X FastDigest Buffer and 1 µl of the FastDigest enzyme (Thermo Scientific). Samples were pulse-vortexed for 15 sec, briefly centrifuged (4000 rpm, 15 sec), then incubated at 37 °C for 5 mins. Using the 2100 Bioanalyzer together with the DNA 1000 LabChip Kit (Agilent Technologies, Böblingen, Germany), participants were classified as *Val/Val*, *Val/Met* or *Met/Met*. The samples were classified based on the observed banding pattern. The uncut product size was 113 bp (*Met/Met*), and *Val/Val* comprised the cut bands of 78 and 35 bp [27,39].

Data analysis

Pre-stimulus *rms*EMG activity was determined in the right and left BB muscles 100 ms prior to each TMS stimulus during pre- and post-testing. Any trial in which pre-stimulus *rms*EMG exceeded 4 ± 1 % of maximal *rms*EMG were discarded, and the trial was repeated. The peak-to-peak amplitude of MEPs evoked as a result of stimulation was measured in the right and left BB muscles contralateral to the cortex being stimulated in the period 10-50 ms after stimulation. MEP amplitudes were analyzed (LabChart 8 software, ADInstruments, Bella Vista, NSW, Australia) after each stimulus was automatically flagged with a cursor, providing peak-to-peak values in µV, averaged and normalized to the M_{MAX}, and multiplied by 100.

Cortical silent period durations were obtained from single-pulse stimuli delivered at 150% and 170% AMT during a light contraction (4% ± 1 of maximal *rms*EMG of the right and left BB muscles). The duration between the onset of the MEP and the resolution of background sEMG was visually inspected and manually cursored, with the experimenter blinded to each condition. The average from 10 stimuli was used for cortical silent period duration [36].

In addition, the laterality index (LI) for interhemispheric asymmetries in corticospinal excitability and inhibition was calculated on the basis of the mean difference in MEP amplitudes between the two hemispheres and the mean difference in cortical silent period duration between the two hemispheres, respectively. In accordance with Cramer et al. [40] and Langan et al. [41], LI was calculated for each condition defined as (L-R)/ (L+R), where L= left hemisphere and R= right hemisphere. A score of 1 reflects complete lateralization to the left side. Conversely, a score of −1 indicates complete lateralization to the right side. In this particular experiment, a positive score indicates greater excitability of the dominant M1 (left hemisphere, right arm).

Statistical analysis

All data were screened with the Shapiro-Wilk test and found to be normally distributed (all $P > 0.05$) and, thus, the assumptions of the ANOVA were not violated. Subsequently, for the primary analysis, a 3 (conditions) x 2 (hemisphere) x 2 (time) repeated measures ANOVA was used to determine any differences between conditions, hemispheres and time for the dependant variables, *rms*EMG, M_{MAX}, corticospinal excitability and cortical silent period duration. If significant main effects were found, a Bonferroni correction was used for *post-hoc* testing to compare the interaction of condition (sham and anodal tDCS) by hemisphere (stimulated and non-stimulated) and time (pre, post) for each dependent variable.

For the secondary analysis, a 2-way ANOVA of genotype (*Val/Val*, *Val/Met*) and time (pre-testing and post-testing) was used to examine the effect of genotype on multiple dependent variables (corticospinal excitability and cortical silent period duration) following anodal tDCS (dominant and non-dominant M1 stimulation arrangements). In addition, paired *t*-tests were performed on VAS

scales and LI score variables. IBM SPSS Statistics 23.0 (United States) was used for all statistical analyses with the level of significance set as $P < 0.05$ for all testing. All data are presented as mean ± SD.

Results

The *BDNF* genotype analysis for the 10 participants for whom we had genetic data revealed that 7 were homozygous for the *Val* allele (*Val66Val*), while 3 were genotyped as *Val66Met*.

Pre-stimulus rmsEMG, active motor threshold stimulus intensity, maximal compound wave, and visual analogue scale

Table 1 presents the mean (± SD) for AMT stimulus intensity, M_{MAX} and single-pulse TMS pre-stimulus *rms*EMG for the stimulated and non-stimulated hemispheres prior to and following sham and anodal tDCS (dominant and non-dominant M1 stimulation). Pre-stimulus *rms*EMG, AMT stimulus intensity and M_{MAX} were similar between sham and anodal tDCS (dominant and non-dominant M1 stimulation) conditions at baseline for each hemisphere (stimulated and non-stimulated; $P > 0.05$). Pre-stimulus *rms*EMG did not vary between single-pulse trials, and there were no TIME, TIME x CONDITION or TIME x CONDITION x HEMISPHERE interactions observed (all $P > 0.05$). Similarly, there was no TIME, TIME x CONDITION or TIME x CONDITION x HEMISPHERE interactions detected for AMT stimulus intensity (all $P > 0.05$). Furthermore, there was no TIME, TIME x CONDITION or TIME x CONDITION x HEMISPHERE interactions detected for M_{MAX}(all $P > 0.05$). VAS data was collected for each condition and there was no difference in participants' perception of discomfort between anodal tDCS (dominant and non-dominant M1 stimulation) and sham conditions (3.31 ± 0.47, 3.23 ± 0.48, 2.80 ± 0.69, respectively; $P = 0.48$).Corticospinal excitability

Corticospinal excitability

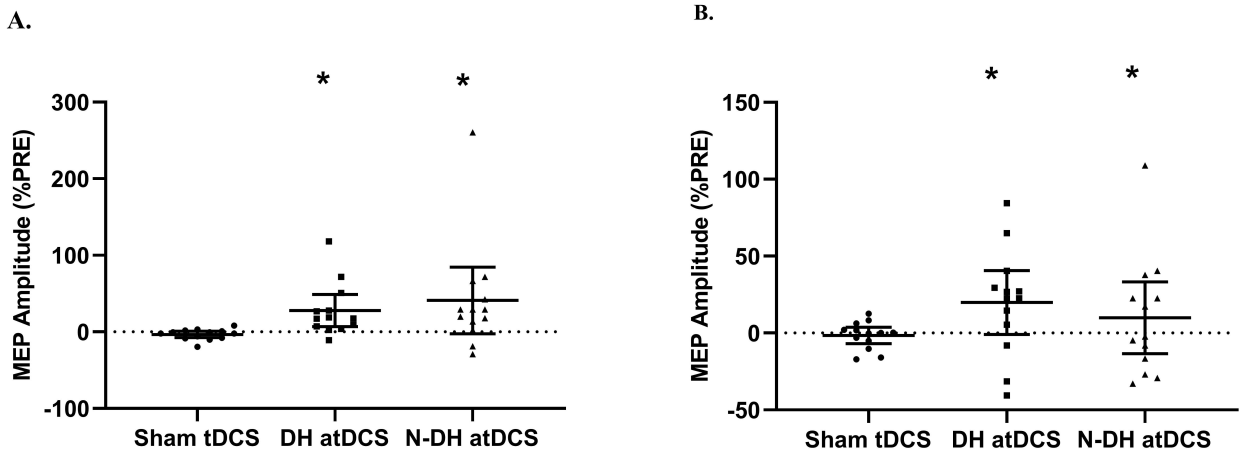

Figure 3. *Mean (± SD) changes in MEP amplitude for the stimulated and non-stimulated hemispheres prior to and following sham, anodal tDCS over the dominant M1 (DH atDCS) and anodal tDCS over the non-dominant M1 (N-DH atDCS) at* ***(A)*** *150% and* ***(B)*** *170% AMT.* * *denotes significant to sham tDCS.*

Figure 3A-B illustrates the percentage change in MEP amplitude for the stimulated and non-stimulated hemispheres following sham and anodal tDCS (dominant and non-dominant M1 stimulation) at 150% (3A) and 170% (3B) of AMT. MEP amplitudes were similar between sham and anodal tDCS (dominant and non-dominant M1 stimulation) conditions at baseline for each hemisphere (stimulated and non-stimulated) and stimulus intensities ($P > 0.05$).

At 150% AMT, there was a main effect for TIME *(P* < 0.001), CONDITION (P = 0.001) and a TIME x CONDITION interaction detected (*P* = 0.001). Importantly, there was no main effect for HEMISPHERE (*P* = 0.816), or TIME x CONDITION x HEMISPHERE interaction (*P* = 0.993) denoting a bilateral increase in MEP amplitude irrespective of which hemisphere was stimulated. *Post hoc* analysis revealed that MEP amplitude increased following anodal tDCS applied over the dominant and non-dominant M1 which was significantly different to sham tDCS (*P* = 0.022; *P* = 0.002, respectively), however this magnitude of change was not different between the dominant and non-dominant M1 (*P* = 0.663, Figure 3A).

Interestingly, the GENOTYPE x TIME ANOVA revealed only a TIME effect at 150% AMT for the *Val/Val* group following anodal tDCS applied over the non-dominant M1 only. Corticospinal excitability increased by 35% for the stimulated right M1 and increased by 40% for the non-stimulated left M1 (*P* < 0.03; *P* = 0.04, respectively). This was compared to a 20% increase and 21% decrease in MEP amplitude in those with the *Val/Met* polymorphism for the stimulated and non-stimulated hemispheres following anodal tDCS of the non-dominant M1. However, *post hoc* analysis revealed that the magnitude of change in MEP amplitude was not statistically significant between genotypes (*P* > 0.05).

At 170% AMT, there was a main effect for TIME (*P* < 0.001), CONDITION (P = 0.009) and a TIME x CONDITION interaction detected (*P* = 0.009). Importantly there was no main effect for HEMISPHERE (*P* = 0.215), or TIME x CONDITION x HEMISPHERE interaction (*P* = 0.062) again denoting a bilateral increase in MEP amplitude irrespective of which hemisphere was stimulated. *Post hoc* analysis revealed that MEP amplitude increased following anodal tDCS applied over the dominant and non-dominant M1 which was significantly different to sham tDCS (*P* = 0.019; *P* = 0.010, respectively), however this magnitude of change was not different between the dominant and non-dominant M1 (*P* = 0.825, Figure 3B).

Interestingly, the GENOTYPE x TIME ANOVA revealed only a TIME effect at 170% AMT for the *Val/Val* group following anodal tDCS applied over the dominant and non-dominant M1 for the stimulated hemisphere (Figure 4A-B, 31%, *P* = 0.03; 50%, *P* = 0.001, respectively). This was compared to a 13% and 31% increase in MEP amplitude in those with the *Val/Met* polymorphism for the stimulated hemisphere following anodal tDCS of the dominant and non-dominant M1. *Post hoc* analysis, however, revealed that the magnitude of change in MEP amplitude was not statistically significant between genotypes (*P* > 0.05).

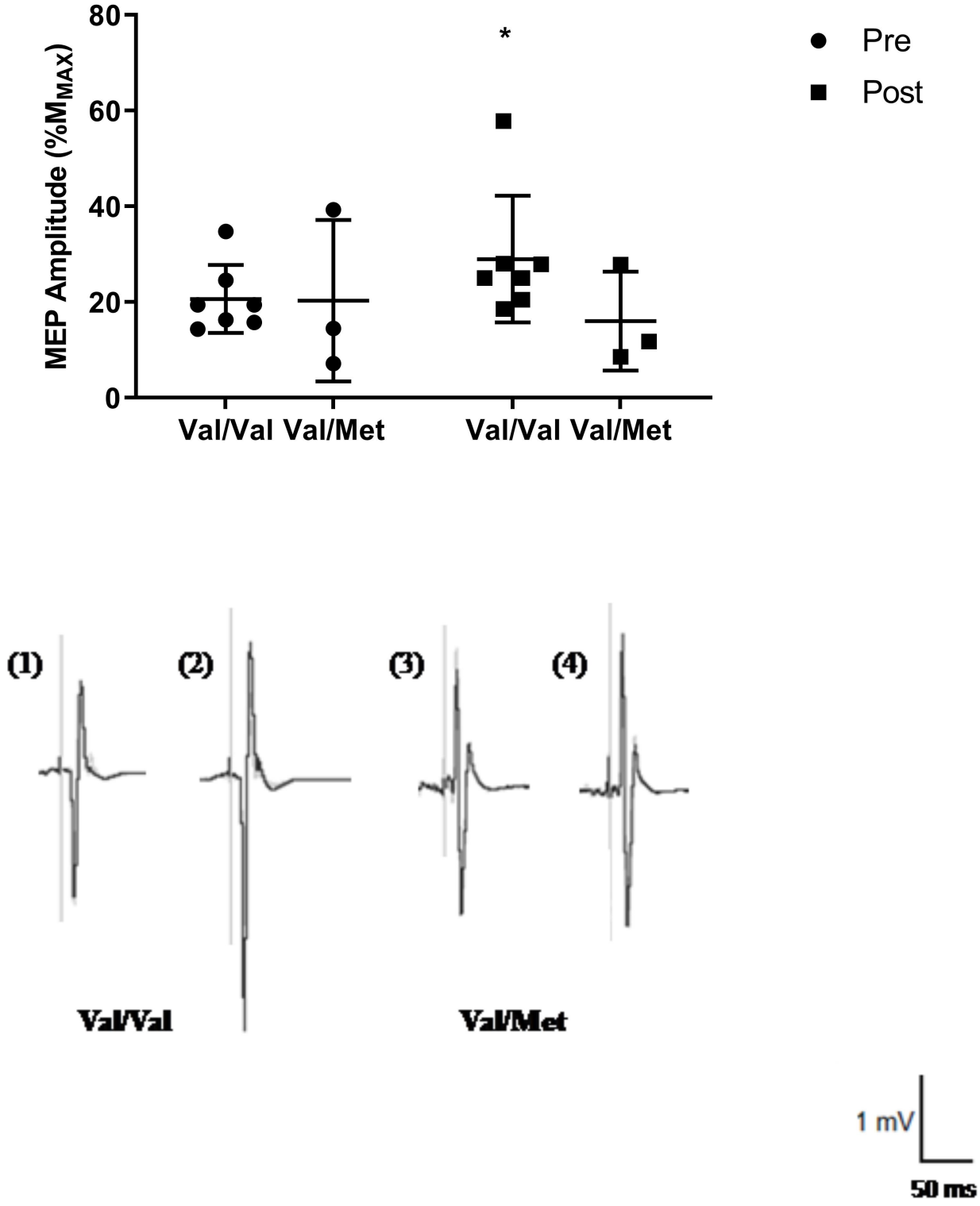

Figure 4. ***(A)*** *Mean (± SD) changes in MEP amplitude of the stimulated hemisphere at 170% AMT following anodal tDCS of the dominant M1 with different BDNF genotypes.* * *denotes significant to baseline.* ***(B)*** *Raw EMG responses (MEPs) of the stimulated hemisphere produced following anodal tDCS of the dominant M1 with different BDNF genotypes, whereby* ***(i)*** *and* ***(ii)*** *depicts pre and post MEP sweeps for the Val/Val individuals,* ***(iii)*** *and* ***(iv)*** *depicts pre and post MEP sweeps for the Val/Met individuals.*

Corticospinal inhibition

Figure 5A-B shows the mean cortical silent period duration for the stimulated and non-stimulated hemispheres prior to and following sham and anodal tDCS of the dominant and non-dominant M1 at 150% and 170% of AMT. Cortical silent period durations were similar between sham and anodal tDCS (dominant and non-dominant M1 stimulation) conditions at baseline for each hemisphere (stimulated and non-stimulated) and stimulus intensities ($P > 0.05$). At 150% AMT, there were no main effects for TIME, TIME x CONDITION or TIME x CONDITION x HEMISPHERE (*all* $P > 0.05$) interactions detected following the intervention (Figure 5A). At 170% AMT, there were a TIME and TIME x CONDITION interaction detected (all $P < 0.05$). *Post hoc* analysis revealed that cortical silent period decreased following anodal tDCS applied over the non-dominant M1 for the non-stimulated hemisphere which was significant to sham tDCS ($P = 0.049$, Figure 5B), however this magnitude of change was not different between the dominant and the non-dominant M1 ($P > 0.05$). Furthermore, there were no TIME ($P > 0.05$) or TIME x CONDITION ($P > 0.05$) interactions detected between genotypes following the intervention.

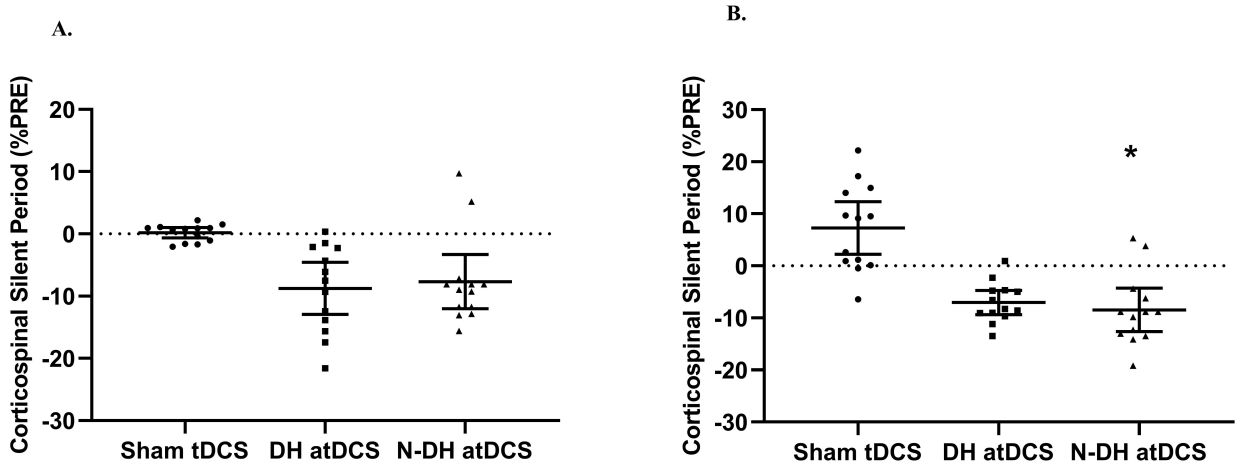

Figure 5. *Mean (± SD) changes in cortical silent period duration for the stimulated and non-stimulated hemispheres prior to and following sham, anodal tDCS over the dominant M1 (DH atDCS) and anodal tDCS over the non-dominant M1 (N-DH atDCS) at* ***(A)*** *150% and* ***(B)*** *170% AMT.* * *denotes significant to sham tDCS.*

Laterality Index

As shown in Table 2, laterality index scores were calculated for each condition for corticospinal excitability and inhibition.

There was no difference in LI detected for corticospinal excitability at 150% AMT for the sham or anodal tDCS conditions (dominant and non-dominant M1 stimulation; $P > 0.05$). There was a significant difference in LI for corticospinal excitability at 170% AMT following anodal tDCS of the non-dominant M1 towards the right (non-dominant) M1 ($P = 0.0047$, Table 2). However, there was no difference in LI detected for the sham condition or following anodal tDCS of the dominant M1 ($P > 0.05$).

There was a significant difference in LI for corticospinal inhibition at 150% AMT following anodal tDCS of the dominant and non-dominant M1 towards the right (non-dominant) M1 ($P = 0.04$; $P = 0.036$, respectively). However, there was no difference in LI detected for the sham condition ($P > 0.05$). Similarly, there was a significant difference in LI for corticospinal inhibition at 170% AMT following anodal tDCS of the dominant and non-dominant M1 towards the right (non-dominant) M1 ($P = 0.02$; $P = 0.018$, respectively) and no difference in LI detected for the sham condition ($P >$

0.05).

	Sham tDCS			DH atDCS			N-DH atDCS	
	Pre	Post		Pre	Post		Pre	Post
MEP (150% AMT)	0.06 ± 0.36	0.06 ± 0.36		0.10 ± 0.25	0.10 ± 0.25		0.06 ± 0.25	0.06 ± 0.21
MEP (170% AMT)	0.06 ± 0.32	0.06 ± 0.21		0.09 ± 0.28	0.08 ± 0.28		0.07 ± 0.28	-0.08 ± 0.28*
SP (150% AMT)	-0.02 ± 0.14	-0.02 ± 0.14		-0.02 ± 0.10	-0.08 ± 010*		-0.03 ± 0.10	-0.07 ± 0.10*
SP (170% AMT)	-0.08 ± 0.07	-0.08 ± 0.07		-0.04 ± 0.07	-0.09 ± 0.10*		-0.04 ± 0.10	-0.08 ± 0.07*

Table 2. *Mean (± SD) for laterality index prior to and following sham and anodal tDCS (dominant and non-dominant hemisphere stimulation arrangements).* DH atDCS: anodal tDCS over the dominant hemisphere; N-DH atDCS: anodal tDCS over the non-dominant hemisphere; MEP: motor evoked potential; SP: silent period; AMT: active motor threshold. * denotes significant to baseline.

Discussion

We investigated the effect of a single session of anodal tDCS on corticospinal excitability and inhibition of both the stimulated and non-stimulated hemisphere following anodal tDCS. In particular, we examined corticospinal excitability/inhibition and the influence of these responses by the *BDNF* polymorphism. The main finding of this study was that a single session of anodal tDCS resulted in a bilateral increase in corticospinal excitability irrespective of which hemisphere (dominant vs non-dominant) was stimulated. In addition, we saw a shift in lateralization of inhibition towards to the right (non-dominant) M1 irrespective of which hemisphere was stimulated. Contrary to our hypothesis, however, we only observed a change in corticospinal inhibition in the non-stimulated hemisphere following anodal tDCS applied over the non-dominant M1. Although we found no significant genotype by time interactions, the within effects for increased corticospinal excitability in the *Val/Val* individuals following anodal tDCS is an important finding that warrants some discussion.

Only a limited number of studies have examined the bilateral effect of NIBS techniques on both the stimulated and non-stimulated hemisphere [13,15,16,18,19]. Interestingly, our finding of increased bilateral corticospinal excitability is not consistent with previous work from Lang et al. [16] who only observed an increase in MEP amplitude in the stimulated hemisphere following anodal tDCS. This difference may lie in methodology as Lang et al. [16] used a protocol of anodal tDCS for 10 min at 1 mA, which may have been insufficient to elicit changes in the contralateral hemisphere (non-stimulated). Importantly our findings are consistent with studies using other NIBS techniques such as rTMS and PAS which have shown bilateral increases in corticospinal excitability [13,18,19]. Our study, however, extends these findings by showing that the magnitude of corticospinal excitability of both the stimulated and non-stimulated hemisphere is irrespective of hemispheric dominance. This finding is noteworthy given that the non-dominant hemisphere has previously been shown to have lower motor threshold, higher MEPs [20] and shorter cortical silent period durations [21], suggesting a greater allowance for the rapid induction of corticospinal plasticity of the non-dominant hemisphere following anodal tDCS. Although in the current study we did not observe any hemispheric differences per se, we have shown a preferential shift of inhibition towards the non-dominant M1. This supports the notion that the non-dominant M1 may be more responsive to anodal tDCS, manifesting as a reduction in the synaptic efficacy between intracortical inhibitory neurons and corticospinal neurons.

Interestingly, we also observed a reduction in cortical silent period duration in the non-stimulated hemisphere following the application of anodal tDCS over the non-dominant M1. This is finding is

notable given that anodal tDCS applied over the non-dominant M1 had no effect on cortical silent period duration in the stimulated hemisphere (right), yet we observed a reduction in cortical silent period duration in the non-stimulated hemisphere (left). Further, there was a similar increase in MEP amplitude between hemispheres (i.e. bilateral increases) which adds further confusion. Although MEP amplitude and cortical silent period duration are independent of each other, changes in cortical inhibition have been proposed to attenuate M1 output via GABA receptor mediated interneuron transmission [42]. At a minimum, the reduction in cortical silent period duration of the non-stimulated left hemisphere following anodal tDCS of the right stimulated hemisphere, shows that there was reduced inhibitory input to the motoneuron pool. Although, it was hypothesised that anodal tDCS of the stimulated hemisphere (irrespective of dominant/non-dominant) would reduce the cortical silent period duration, the result that anodal tDCS of the right hemisphere had no effect on the cortical silent period, was surprising. Although the cortical projections to the biceps brachii are less divergent than other upper limb muscle groups [43] , this may suggest the potential to undergo plasticity following anodal tDCS may have been limited. A caveat to this interpretation, however, is confounded by the fact that there was only a reduction in cortical silent period duration for the non-stimulated hemisphere, following stimulation of the right M1. Despite this, given we have shown bilateral increases in corticospinal excitability, a single session of anodal tDCS appears to modulate mechanisms associated with long-term potentiation (LTP) rather than neurons that use gamma-aminobutyric acid ($GABA_B$) as their neurotransmitters. The after-effects of tDCS are well established, with the general consensus that the mechanisms underlying corticospinal plasticity are due to changes in synaptic strength due to modulation of the NMDA receptor [10,22]. This experiment provides evidence that anodal tDCS not only improves synaptic efficacy of the stimulated hemisphere, but also modulates corticospinal connections of the non-stimulated hemisphere. One possible explanation is that anodal tDCS of the stimulated hemisphere leads to a reduction in IHI of the non-stimulated hemisphere and consequently increases corticospinal excitability of the non-stimulated hemisphere [13]. A caveat to this interpretation is that IHI was not measured and thus we do not feel able to draw any definitive conclusion regarding potential underlying mechanisms. However, given that fMRI studies in humans have shown anodal tDCS to activate extended neural networks [44] and reduce transcallosal inhibition [16], we suggest that it is plausible that anodal tDCS acts on both excitatory and inhibitory synaptic inputs which may shift the balance in excitability between hemispheres. Furthermore, we saw a small but significant shift in lateralization of inhibition towards the right (non-dominant) M1 irrespective of which hemisphere was stimulated. Although we are uncertain as to why this has occurred, and how this may affect motor performance, it may have relevance to rehabilitation following pathology to the right M1. However, given that we are unsure of the underlying mechanisms causing this shift in lateralization, we can only speculate as to the potential implications of this finding and further experiments would be required to resolve this point of discussion.

It appears that the *BDNF* polymorphism shaped the induction of corticospinal plasticity following a single session of anodal tDCS. Recent data has shown that carriers of the *BDNF Met* allele (*Val/Met*) display reduced corticospinal responses following repeated bouts of anodal tDCS compared to the *Val/Val* genotype [27]. Similarly, we found that when individuals were sub-grouped into genotype, and individual data was examined, the *Val/Val* individuals showed a greater increase in MEP amplitude compared to *Val/Met* individuals. This trend was evident for both the dominant and non-dominant M1, irrespective of which hemisphere was stimulated, however, this magnitude was not statistically significant due to the limited sample size of the *Val/Met* group. Importantly, this data provides further insight into the important role that the *BDNF* polymorphism plays in the induction of experimentally-induced plasticity, and that this effect may be evident from as little as 20 min of anodal tDCS.

These findings show that a single session of anodal tDCS induced bilateral effects in corticospinal excitability, irrespective of which hemisphere was stimulated (dominant vs non-dominant). The induction of corticospinal plasticity appears to be influenced by the *BDNF* polymorphism; however this notion should be interpreted with caution give the small sample size, indicating the need for future investigation.

Disclosure Statement

None of the authors have potential conflicts of interest to be disclosed.

List of Abbreviations

AMT: active motor threshold

BB: biceps brachii

***BDNF*:** brain-derived neurotrophic factor

IHI: interhemispheric inhibition

iTMS:theta burst stimulation

LTP: long-term potentiation

MEPs: motor-evoked potentials

MVIC: maximal voluntary isometric contraction

M1: primary motor cortex

NMDA: N-Methyl-D-aspartate

NIBS: non-invasive brain stimulation

PAS: paired associative stimulation

***rms*EMG:** root-mean square electromyography

rTMS: repetitive transcranial magnetic stimulation

sEMG: surface electromyography

tDCS: transcranial direct current stimulation

TMS: transcranial magnetic stimulation

VAS: visual analogue scale

References

1. Roy Abhrajeet, Baxter Bryan, Bin He. High-Definition Transcranial Direct Current Stimulation Induces Both Acute and Persistent Changes in Broadband Cortical Synchronization: A Simultaneous tDCS–EEG Study. *IEEE Transactions on Biomedical Engineering.* 2014; 61(7)DOI
2. Nitsche M. A., Doemkes S., Karaköse T., Antal A., Liebetanz D., Lang N., Tergau F., Paulus W.. Shaping the Effects of Transcranial Direct Current Stimulation of the Human Motor Cortex. *Journal of Neurophysiology.* 2007; 97(4)DOI
3. Nitsche M. A., Paulus W.. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology.* 2000; 527(3)DOI

4. Nitsche Michael A., Cohen Leonardo G., Wassermann Eric M., Priori Alberto, Lang Nicolas, Antal Andrea, Paulus Walter, Hummel Friedhelm, Boggio Paulo S., Fregni Felipe, Pascual-Leone Alvaro. Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation.* 2008; 1(3)DOI
5. Rahman Asif, Reato Davide, Arlotti Mattia, Gasca Fernando, Datta Abhishek, Parra Lucas C., Bikson Marom. Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. *The Journal of Physiology.* 2013; 591(10)DOI
6. Bastani A., Jaberzadeh S.. Does anodal transcranial direct current stimulation enhance excitability of the motor cortex and motor function in healthy individuals and subjects with stroke: A systematic review and meta-analysis. *Clinical Neurophysiology.* 2012; 123(4)DOI
7. Kidgell Dawson J, Goodwill Alicia M, Frazer Ashlyn K, Daly Robin M. Induction of cortical plasticity and improved motor performance following unilateral and bilateral transcranial direct current stimulation of the primary motor cortex. *BMC Neuroscience.* 2013; 14(1)DOI
8. Nitsche M. A., Paulus W.. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology.* 2001; 57(10)DOI
9. Pellicciari Maria Concetta, Brignani Debora, Miniussi Carlo. Excitability modulation of the motor system induced by transcranial direct current stimulation: A multimodal approach. *NeuroImage.* 2013; 83DOI
10. Nitsche Michael A., Seeber Antje, Frommann Kai, Klein Cornelia Carmen, Rochford Christian, Nitsche Maren S., Fricke Kristina, Liebetanz David, Lang Nicolas, Antal Andrea, Paulus Walter, Tergau Frithjof. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. *The Journal of Physiology.* 2005; 568(1)DOI
11. Liebetanz D.. Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain.* 2002; 125(10)DOI
12. Nitsche M. A., Fricke K., Henschke U., Schlitterlau A., Liebetanz D., Lang N., Henning S., Tergau F., Paulus W.. Pharmacological Modulation of Cortical Excitability Shifts Induced by Transcranial Direct Current Stimulation in Humans. *The Journal of Physiology.* 2003; 553(1)DOI
13. Gilio Francesca, Rizzo Vincenzo, Siebner Hartwig R., Rothwell John C.. Effects on the right motor hand-area excitability produced by low-frequency rTMS over human contralateral homologous cortex. *The Journal of Physiology.* 2003; 551(2)DOI
14. Di Lazzaro V., Dileone M., Pilato F., Capone F., Musumeci G., Ranieri F., Ricci V., Bria P., Di Iorio R., de Waure C., Pasqualetti P., Profice P.. Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. *Journal of Neurophysiology.* 2011; 105(5)DOI
15. Di Lazzaro V., Pilato F., Dileone M., Profice P., Oliviero A., Mazzone P., Insola A., Ranieri F., Meglio M., Tonali P. A., Rothwell J. C.. The physiological basis of the effects of intermittent theta burst stimulation of the human motor cortex. *The Journal of Physiology.* 2008; 586(16)DOI
16. Lang N., Nitsche M. A., Paulus W., Rothwell J. C., Lemon R. N.. Effects of transcranial direct current stimulation over the human motor cortex on corticospinal and transcallosal excitability. *Experimental Brain Research.* 2004; 156(4)DOI
17. Suppa A., Ortu E., Zafar N., Deriu F., Paulus W., Berardelli A., Rothwell J. C.. Theta burst stimulation induces after-effects on contralateral primary motor cortex excitability in humans. *The Journal of Physiology.* 2008; 586(18)DOI
18. Schambra H.M, Sawaki L, Cohen L.G. 0.1097/WNR.0b013e3283439511Modulation of excitability of human motor cortex (M1) by 1 Hz transcranial magnetic stimulation of the contralateral M1. *Clinical Neurophysiology.* 2003; 114(1)DOI
19. Shin Hae-Won, Sohn Young H.. Interhemispheric transfer of paired associative stimulation-induced plasticity in the human motor cortex. *NeuroReport.* 2011; 22(4)DOI
20. De Gennaro Luigi, Cristiani Riccardo, Bertini Mario, Curcio Giuseppe, Ferrara Michele, Fratello Fabiana, Romei Vincenzo, Rossini Paolo M. Handedness is mainly associated with an asymmetry of corticospinal excitability and not of transcallosal inhibition. *Clinical Neurophysiology.* 2004; 115(6)DOI

21. Priori A., Oliviero A., Donati E., Callea L., Bertolasi Laura, Rothwell J. C.. Human handedness and asymmetry of the motor cortical silent period. *Experimental Brain Research.* 1999; 128(3)DOI
22. Boggio Paulo S., Castro Letícia O., Savagim Edna A., Braite Renata, Cruz Viviane C., Rocha Renata R., Rigonatti Sergio P., Silva Maria T.A., Fregni Felipe. Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation. *Neuroscience Letters.* 2006; 404(1-2)DOI
23. Tanaka Satoshi, Hanakawa Takashi, Honda Manabu, Watanabe Katsumi. Enhancement of pinch force in the lower leg by anodal transcranial direct current stimulation. *Experimental Brain Research.* 2009; 196(3)DOI
24. Vines Bradley W., Nair Dinesh G., Schlaug Gottfried. Contralateral and ipsilateral motor effects after transcranial direct current stimulation. *NeuroReport.* 2006; 17(6)DOI
25. Hendy Ashlee M., Kidgell Dawson J.. Anodal-tDCS applied during unilateral strength training increases strength and corticospinal excitability in the untrained homologous muscle. *Experimental Brain Research.* 2014; 232(10)DOI
26. Ruddy Kathy L., Carson Richard G.. Neural pathways mediating cross education of motor function. *Frontiers in Human Neuroscience.* 2013; 7DOI
27. Frazer Ashlyn, Williams Jacqueline, Spittles Michael, Rantalainen Timo, Kidgell Dawson. Anodal transcranial direct current stimulation of the motor cortex increases cortical voluntary activation and neural plasticity. *Muscle & Nerve.* 2016; 54(5)DOI
28. Hwang Jung Min, Kim Yun-Hee, Yoon Kyung Jae, Uhm Kyeong Eun, Chang Won Hyuk. Different responses to facilitatory rTMS according to BDNF genotype. *Clinical Neurophysiology.* 2015; 126(7)DOI
29. Puri Rohan, Hinder Mark R., Fujiyama Hakuei, Gomez Rapson, Carson Richard G., Summers Jeffery J.. Duration-dependent effects of the BDNF Val66Met polymorphism on anodal tDCS induced motor cortex plasticity in older adults: a group and individual perspective. *Frontiers in Aging Neuroscience.* 2015; 7DOI
30. Chang Won Hyuk, Bang Oh Young, Shin Yong-Il, Lee Ahee, Pascual-Leone Alvaro, Kim Yun-Hee. BDNF Polymorphism and Differential rTMS Effects on Motor Recovery of Stroke Patients. *Brain Stimulation.* 2014; 7(4)DOI
31. Oldfield R.C.. The assessment and analysis of handedness: The Edinburgh inventory. *Neuropsychologia.* 1971; 9(1)DOI
32. Keel John C, Smith Mark J, Wassermann Eric M. A safety screening questionnaire for transcranial magnetic stimulation. *Clinical Neurophysiology.* 2001; 112(4)DOI
33. Vines Bradley W, Cerruti Carlo, Schlaug Gottfried. Dual-hemisphere tDCS facilitates greater improvements for healthy subjects' non-dominant hand compared to uni-hemisphere stimulation. *BMC Neuroscience.* 2008; 9(1)DOI
34. Griffin L., Cafarelli E.. Transcranial magnetic stimulation during resistance training of the tibialis anterior muscle. *Journal of Electromyography and Kinesiology.* 2007; 17(4)DOI
35. Pearce A. J., Hendy A., Bowen W. A., Kidgell D. J.. Corticospinal adaptations and strength maintenance in the immobilized arm following 3 weeks unilateral strength training. *Scandinavian Journal of Medicine & Science in Sports.* 2012; 23(6)DOI
36. Wilson S.A., Lockwood R.J., Thickbroom G.W., Mastaglia F.L.. The muscle silent period following transcranial magnetic cortical stimulation. *Journal of the Neurological Sciences.* 1993; 114(2)DOI
37. Hendy Ashlee M., Teo Wei-Peng, Kidgell Dawson J.. Anodal Transcranial Direct Current Stimulation Prolongs the Cross-education of Strength and Corticomotor Plasticity. *Medicine & Science in Sports & Exercise.* 2015; 47(9)DOI
38. Ziemann Ulf, Paulus Walter, Nitsche Michael A., Pascual-Leone Alvaro, Byblow Winston D., Berardelli Alfredo, Siebner Hartwig R., Classen Joseph, Cohen Leonardo G., Rothwell John C.. Consensus: Motor cortex plasticity protocols. *Brain Stimulation.* 2008; 1(3)DOI
39. Neves-Pereira Maria, Mundo Emanuela, Muglia Pierandrea, King Nicole, Macciardi Fabio, Kennedy James L.. The Brain-Derived Neurotrophic Factor Gene Confers Susceptibility to Bipolar Disorder: Evidence from a Family-Based Association Study. *The American Journal of Human Genetics.* 2002; 71(3)DOI

40. Cramer Steven C., Nelles Gereon, Benson Randall R., Kaplan Jill D., Parker Robert A., Kwong Ken K., Kennedy David N., Finklestein Seth P., Rosen Bruce R.. A Functional MRI Study of Subjects Recovered From Hemiparetic Stroke. *Stroke.* 1997; 28(12)DOI
41. Langan. Functional implications of age differences in motor system connectivity. *Frontiers in Systems Neuroscience.* 2010. DOI
42. McCormick D. A.. GABA as an inhibitory neurotransmitter in human cerebral cortex. *Journal of Neurophysiology.* 1989; 62(5)DOI
43. Palmer E, Ashby P. Corticospinal projections to upper limb motoneurones in humans.. *The Journal of Physiology.* 1992; 448(1)DOI
44. Krishnamurthy Venkatagiri, Gopinath Kaundinya, Brown Gregory S., Hampstead Benjamin M.. Resting-state fMRI reveals enhanced functional connectivity in spatial navigation networks after transcranial direct current stimulation. *Neuroscience Letters.* 2015; 604DOI