Anodal transcranial direct current stimulation of the motor cortex increases cortical voluntary activation and neural plasticity

Anodal Transcranial Direct Current Stimulation of the Motor Cortex Increases Cortical Voluntary Activation and Neural Plasticity.

Ashlyn Frazer, BEx&SS (Hons)[1], Jacqueline Williams, PhD[1], Michael Spittles, PhD[1], Timo Rantalainen, PhD[2], Dawson Kidgell, PhD[3]

[1]Institute of Sport, Exercise and Active Living, Victoria University, Melbourne, Australia.

[2]Centre for Physical Activity and Nutrition Research, Deakin University, Melbourne, Australia.

[3]Department of Rehabilitation, Nutrition and Sport, School of Allied Health, La Trobe University, Melbourne, Australia.

Address for Correspondence and Reprints:

Dawson J Kidgell, PhD

Department of Rehabilitation, Nutrition and Sport

School of Allied Health

La Trobe University, Melbourne, Australia

Phone: +61 3 9479 3713

Email: D.Kidgell@latrobe.edu.au

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ABSTRACT

Introduction: We examined the cumulative effect of 4 consecutive bouts of non-invasive brain stimulation on corticospinal plasticity and motor performance, and whether these responses were influenced by the brain-derived neurotrophic factor (*BDNF*) polymorphism. ***Methods:*** In a randomized double-blinded cross-over design, changes in strength and indices of corticospinal plasticity were analyzed in 14 adults who were exposed to 4 consecutive sessions of anodal and sham transcranial direct current stimulation (tDCS). Participants also undertook a blood sample for *BDNF* genotyping (N=13). ***Results:*** We observed a significant increase in isometric wrist flexor strength with transcranial magnetic stimulation revealing increased corticospinal excitability, decreased silent period duration, and increased cortical voluntary activation compared to sham tDCS. ***Discussion:*** The results show that 4 consecutive sessions of anodal tDCS increased cortical voluntary activation manifested as an improvement in strength. Induction of corticospinal plasticity appears to be influenced by the *BDNF* polymorphism.

Key words: *BDNF* polymorphism, cortical voluntary activation, motor performance, neural plasticity, strength, transcranial direct current stimulation.

INTRODUCTION

The excitability of cortical neurons in the primary motor cortex (M1) can be readily modified by application of weak transcranial direct currents, which leads to induction of M1 plasticity. In particular, transcranial direct current stimulation (tDCS) of the M1 elicits changes in cortical excitability in a polarity-specific manner when measured by transcranial magnetic stimulation (TMS). In general, anodal tDCS induces facilitatory effects on motor-evoked potentials (MEPs), while cathodal tDCS leads to inhibitory effects.[1] Specifically, following a single session tDCS with current intensities of 0.6 mA to 2 mA applied for 5–20 minutes has been shown to modulate cortical excitability for up to 90 minutes after stimulation.[1-5]

This temporary modification in cortical plasticity following anodal-tDCS has been reported to correspond with transient improvements in motor performance.[4, 6-10] For example, following a single session of tDCS (in the absence of motor training), improved motor performance in tasks such as the Jebsen-Taylor hand function test, maximal strength of the elbow flexors and knee extensors, the Purdue pegboard test, maximal pinch force, reaction time, and tests of motor sequencing tasks have all been reported. [4, 6-10] In a similar context, in healthy adults, repeated sessions of tDCS has also been shown to improve motor performance (Jebsen-Taylor hand function test and sequential visual isometric pinch task), with retention lasting up to 3 months following stimulation.[11, 12] Despite this evidence, a recent systematic review and meta-analysis reported that the effects of a single session of anodal tDCS did not show any statistically significant difference for motor function in healthy participants or stroke patients.[3]

Following central nervous system injury, such as stroke, there is reduced neural drive to the affected muscle, which produces reduced voluntary activation.[13] Deficits in voluntary

activation have traditionally been assessed with the interpolated twitch technique. Briefly, twitch interpolation involves application of a single supramaximal electrical stimulus to the corresponding motor nerve during a maximum voluntary isometric contraction (MVIC). If the supramaximal electrical stimulus fails to produce additional force during the MVIC, it has been suggested that the muscle force produced voluntarily is maximal, and voluntary activation is complete.[14, 15] On the other hand, if extra force is evoked during supramaximal stimulation, voluntary activation may be incomplete. Voluntary activation is determined by comparing the size of the evoked twitch force (superimposed twitch force) with the force that is produced by the same stimulus intensity at rest (resting twitch force). Several studies [14, 15] have shown that incomplete voluntary activation (using twitch interpolation) manifests as a reduction in the 'voluntary' force generating capacity of the muscle due to reduced neural drive at or above the site of stimulation of the motor nerve. A potential limitation of twitch interpolation is that it fails to define the site of neural drive impairment.[16] Thus, more recently, TMS has been used to assess 'cortical' voluntary activation.[17] However, unlike twitch interpolation, the presence of a superimposed twitch force produced by a suprathreshold TMS pulse during an MVIC indicates a failure in neural drive at the level of the motor cortex.[16]

Interestingly, although previous studies have shown that anodal tDCS applied over the leg motor cortex improves force production [9, 10], no studies have examined the effects of repeated sessions of anodal tDCS on muscle strength and cortical voluntary activation. Furthermore, recovery from neuromuscular injury often requires induction of neural plasticity within the M1[18], however in humans there is a single nucleotide polymorphism of the *BDNF* gene (*BDNF Val66Met*) that results in reduced *BDNF* release in cortical neurons.[19] Recently, it has been shown that induction of M1 plasticity, assessed with TMS, is reduced in both experimentally-induced (e.g. rTMS, tDCS) and use-dependant M1 plasticity (e.g. motor

learning) in participants with the *BDNF* polymorphism.[20, 21] For example, induction of plasticity following non-invasive brain stimulation (NIBS) techniques such as rTMS has been shown to be differentially modulated based on the *BDNF* polymorphism.[22,23] But relevant to this study, only 1 study has investigated the impact of the *BDNF* polymorphism on M1 plasticity induced by a single session of anodal and cathodal tDCS.[21] Interestingly, a similar finding has also been observed in older adults following anodal tDCS.[23] Critically, to our knowledge, there are no studies of whether the *BDNF* polymorphism influences cortical voluntary activation or the expression for muscle force following accumulated bouts of anodal tDCS.

Therefore, we examined the effect of repeated sessions of anodal tDCS on muscle strength, cortical voluntary activation, and indices of corticospinal M1 plasticity. In particular, we examined corticospinal excitability/inhibition and the influence on these responses by the *BDNF* polymorphism. We hypothesized that induction of experimentally-induced M1 plasticity (increased cortical excitability and reduced cortical inhibition) would improve muscle strength and cortical voluntary activation, but the magnitude of these responses would be influenced by the *BDNF* polymorphism.

EXPERIMENTAL PROCEDURES

Participants

Fourteen participants (8 women, 6 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[24] with an LQ score of 83 ± 5, had not participated in

strength training for at least 12 months, 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.[25]

Experimental approach

Figure 1 outlines the organization of the study. After obtaining consent, participants completed a familiarization session 1 week prior to the study that involved performing 5 isometric contractions of the right wrist flexors and extensors and were exposed to single-pulse TMS to reduce any potential learning effect. In a double-blinded cross-over design, all participants were exposed to 4 days of anodal and sham tDCS. The order of the conditions was counterbalanced and randomized between participants, with a wash-out period of 1 week between each condition.[26] Both tDCS conditions followed the identical testing protocol as shown in Figure 1. All participants underwent TMS and isometric strength testing (MVIC) of the right wrist flexors and extensors prior to and following the tDCS intervention. Participants were required to attend 4 sessions on consecutive days where they were exposed to 20 min of anodal or sham tDCS applied at 2 mA. Post-testing was carried out 24 hours after the final tDCS session.

Voluntary strength testing

MVIC of the right wrist flexors and extensors was determined on a custom-made force transducer (Futek Force Transducer LSB302, Melbourne). For the wrist flexor MVIC, participants were seated in a chair, shoulders relaxed with their elbows flexed at 110 degrees. With the hand supinated and the force transducer 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. For wrist extensors MVIC, the forearm was pronated, and the participant was instructed to extend the dorsum of their hand as forcefully as possible against

the force-transducer. Three trials were performed; each trial was 3 sec in duration, separated by 3 min rest to minimize fatigue. The greatest recorded output was recorded as the participant's MVIC for the wrist flexors and extensors.

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 flexor carpi radialis (FCR) and right extensor carpi radialis (ECR) muscles using bipolar Ag-AgCl electrodes. As described by Selvanayagam et al.[27] the electrodes for the FCR were positioned 9 cm from the medial epicondyle of the humerus with an inter-electrode distance (center to center) of 2 cm. The ECR electrodes were positioned at 45% of the distance from the medial epicondyle of the humerus to the radial styloid process with an inter-electrode distance of 2 cm. A grounding strap was placed around the wrist as the common reference point for all electrodes. 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 and cortical voluntary activation

TMS was delivered using 2 Magstim 200^2 stimulators (Magstim Co, Dyfed, UK) connected via a Bistim unit and a single figure-of-eight coil (external diameter of each loop 70 mm). The motor hotspot for the FCR (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 FCR muscle. 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.

All stimuli were delivered during a low level isometric contraction of the wrist flexors, which were performed on the custom-made force transducer and involved supinating the hand and maintaining 110 degrees of elbow flexion. Using a horizontal line on the computer screen as visual feedback, participants were instructed to hold 2% of the wrist flexors MVIC which equated to 0.97 ± 0.09 % of the maximal root-mean squared electromyography (rmsEMG), which was obtained during MVIC testing. Consistent muscle activation was confirmed by recording pre-stimulus rmsEMG for the 100 ms epoch prior to the delivery of each stimulus (see Table 1). To control for background sEMG prior to TMS stimulation, all MEPs obtained during isometric contractions post-intervention were obtained at the pre-force level.

To quantify short interval intracortical inhibition (SICI), 5 single-pulse stimuli and 5 short-interval paired-pulse stimuli were delivered in a random order.[28] The stimulator output intensity was set at 120% AMT, which was determined during familiarization and adjusted if there was a change following tDCS. The conditioning stimulus for paired-pulse stimulation was set at 80% AMT, the inter-stimulus interval was 3 ms, and subsequent posterior to anterior current flow was used.[29,30]

In accordance with Lee et al.[31], voluntary activation was calculated using an average of 3 trials. Each trial consisted of 3 isometric wrist contractions (3 sec) with a 2 min rest between trials. Participants were instructed to match a required force (50, 75, and 100% of wrist flexors MVIC) using a horizontal line on the computer screen as visual feedback. TMS was delivered over the contralateral M1 to evoke superimposed twitches during voluntary contractions. The TMS stimulus intensity for each subject was determined by MEP_{MAX} which was identified from the stimulus response curve. This stimulus intensity corresponded to at least 50% M_{MAX} of the wrist flexors and a relatively small MEP (< 10% M_{MAX}) of the wrist extensors.

Maximum compound muscle action potential

Direct muscle responses were obtained from the right FCR and ECR muscles by supramaximal electrical stimulation (pulse width 200 μs) of the median and radial nerves, respectively, while holding 2% of the wrist flexors and extensors MVIC, which equated to 0.97 ± 0.09 % of the maximal rmsEMG (DS7A, Digitimer, UK). This low level of muscle activity was used to match the conditions under which TMS was delivered. The stimulation site that produced the largest M-wave was located by positioning the bipolar electrodes in the cubital fossa (median nerve) and on the lateral aspect of the arm above the elbow (radial nerve). An increase in current strength was applied to the median and radial nerves 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 5 stimuli, with a period of 6-9 sec 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 for 4 consecutive days 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 [32]), each soaked in saline solution (0.9% NaCl) and secured on the head with a rubber strap. Anodal tDCS involved 20 min at 2 mA stimulation intensity with the anode fixed over the optimal cortical representation of the right FCR muscle, as identified by TMS over the left cortex, and the cathode was placed over the right contralateral supra orbital area. 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 through the use of codes on the tDCS machine. Using the protocol suggested by the international consensus paper on NIBS techniques[33], the sham protocol had the identical arrangement to the anodal tDCS condition, but the stimulation terminated after approximately 20 sec. This resulted in the participant experiencing the initial sensation of tDCS, however no experimental effects occurred. In order to obtain the participant's perception of discomfort throughout both tDCS conditions, discomfort (which included pain, itching, and tingling sensations) was assessed using a visual analogy 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

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.

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 (Mgcl2) (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.[34], 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.[34]

Data analysis

Pre-stimulus rmsEMG activity was determined in the right wrist flexors 100 ms prior to each TMS stimulus during pre- and post-testing. Any trial in which pre-stimulus rmsEMG exceeded 1 ± 0.5 % of maximal rmsEMG were discarded, and the trial was repeated. The peak-to-peak amplitude of MEPs evoked as a result of stimulation was measured in the FCR muscle 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.

The conditioned MEP amplitude was expressed as a percentage of the unconditioned test MEP amplitude to calculate the level of SICI. Silent period durations were obtained from single-pulse stimuli delivered at 110-210% AMT (increments of 20%) during a light contraction (2% of the wrist flexors MVIC which equated to 0.97 ± 0.09 % of the maximal rmsEMG). All post- measures were obtained at the pre-force level, as increases in background sEMG as a result of the tDCS intervention could confound MEP amplitudes. 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 8 stimuli was used for silent period duration.[35]

To calculate cortical voluntary activation, a linear regression of the amplitude of the superimposed twitch was plotted against voluntary force levels of 50, 75, and 100% of the wrist flexors MVIC for each participant to determine the level of cortical voluntary activation. Cortical voluntary activation was calculated using a linear regression between the superimposed twitches and the voluntary maximal force, whereby the y-intercept was taken

as the estimated resting twitch. Cortical voluntary activation was calculated using the following equation:

Cortical voluntary activation % = (1-superimposed twitch force/estimated resting twitch force) x 100.[16, 17, 31]

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 split-plot in time, repeated measure ANOVA was used to compare the effects of anodal and sham tDCS conditions on multiple dependent variables (MVIC force, pre-stimulus EMG, corticospinal excitability, SICI, silent period, and voluntary cortical voluntary activation) over 2 time points (pre-testing and post-testing). 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 (MVIC force, corticospinal excitability, SICI, silent period, and cortical voluntary activation) following anodal tDCS. Bonferroni correction for multiple comparisons was applied for each dependent variable where significant multivariate effects were found. Prism 6 for Windows (Graphpad Software Inc, CA, USA) 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 ± SE.

RESULTS

The *BDNF* genotype analysis for the 13 participants for whom we had genetic data revealed that 10 were homozygous for the *Val* allele (*Val66Val*), while 3 were genotyped as *Val66Met*.

Pre-stimulus rmsEMG, maximal compound wave, and visual analogue scale

Pre-stimulus rmsEMG did not vary between single- and paired-pulse trials, and there were no TIME ($P > 0.05$) or TIME x CONDITION ($P > 0.05$) interactions observed. Similarly, no TIME ($P > 0.05$) or TIME x CONDITION ($P > 0.05$) interactions were detected for M_{MAX} (Table 1). VAS data were pooled across 4 sessions for each condition, and there was no difference in participants' perception of discomfort between sham and anodal tDCS conditions (2.85 ± 0.27, 2.88 ± 0.23 respectively; $P = 0.93$).

Maximal voluntary isometric contraction

Isometric strength was assessed for the right wrist flexors and extensors prior to and following 4 consecutive sessions of sham and anodal tDCS. Figure 2 shows the mean change in isometric strength for the right wrist flexors. There were no differences in isometric strength at baseline for the wrist flexors and extensors between sham and anodal conditions ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P = 0.01$) and a TIME x CONDITION interaction ($P = 0.02$) for an increase in isometric wrist flexor strength. *Post hoc* analysis showed that anodal tDCS stimulation resulted in an 8% increase in isometric wrist flexor strength compared to 3% following sham tDCS. However, there was no difference in isometric wrist flexors strength between genotypes ($P > 0.05$). Furthermore, no TIME ($P > 0.05$) or TIME x CONDITION ($P > 0.05$) interactions were detected for isometric wrist extensor strength following the intervention.

Corticospinal excitability

Figure 3A-B shows the mean MEP amplitude normalized as a percentage of M_{MAX} for anodal and sham tDCS conditions at 110-210% of AMT (increments of 20%) of the wrist flexors. MEP amplitudes were similar between sham and anodal tDCS conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention, there was a main effect for TIME ($P < 0.05$) and a TIME x CONDITION interaction ($P < 0.05$) at all stimulus intensities (110-210% AMT). *Post hoc* analysis showed that anodal tDCS stimulation resulted in a 32-67% increase in MEP amplitude across 110-210% of AMT compared to an average of 1-9% change in the sham tDCS condition (Table 2). Interestingly, the GENOTYPE x TIME ANOVA revealed only a TIME effect for the *Val/Val* group at 110%, 130%, and 150% AMT ($P < 0.05$; Figure 3C). At 110% AMT, MEP amplitude increased by 60% compared to a 48% increase in those with the *Val/Met* polymorphism. At 130% AMT, *Val/Val* individuals increased their mean MEP amplitude by 68% compared to a 26% increase in those with the *Val/Met* polymorphism. A similar effect was observed at 150% AMT, with *Val/Val* individuals increasing mean MEP amplitude by 55% compared to a 4% increase in those with the *Val/Met* polymorphism. However, *post hoc* analysis revealed that the magnitude of change in MEP amplitude was not statistically significant between genotypes ($P > 0.05$).

There were no differences in MEP amplitudes across all stimulus intensities (110-210% AMT; increments of 20%) of the wrist extensors between groups at baseline ($P > 0.05$). There were no main effects for TIME ($P > 0.05$) or CONDITION x TIME ($P > 0.05$) interactions detected following the intervention. Furthermore, there were no TIME ($P > 0.05$) or TIME x CONDITION ($P > 0.05$) interactions detected between genotypes following the intervention.

Corticospinal inhibition

As shown in Figure 4A-B, corticospinal inhibition was assessed with the duration of the silent period obtained at a number of stimulus intensities above AMT (110-210% AMT; increments of 20%). Silent period durations were similar between sham and anodal tDCS conditions at baseline across all stimulus intensities ($P > 0.05$). Following the intervention there was a main effect for TIME ($P < 0.05$) and a CONDITION x TIME interaction ($P < 0.05$) at a 130-210% of AMT. *Post hoc* analysis showed that anodal tDCS resulted in a 8-12% decrease in silent period duration compared to an average of 1% decrease in the sham tDCS condition. Interestingly, the GENOTYPE x TIME ANOVA revealed only a TIME effect for the *Val/Val* group at 130% and 150% of AMT ($P < 0.05$; Figure 4C). At 130% of AMT, silent period duration reduced by 14% following anodal tDCS in the *Val/Val* group compared to a 3% reduction in those with the *Val/Met* polymorphism. A similar effect was observed at 150% AMT, with *Val/Val* individuals reducing silent period duration by 17% compared to a 6% reduction in those with the *Val/Met* polymorphism. However, *post hoc* analysis revealed that the magnitude of change in silent period duration was not statistically significant between genotypes ($P > 0.05$).

Short-interval intracortical inhibition

There were no differences in SICI between groups at baseline ($P > 0.05$). There were no main effects for TIME ($P = 0.55$) or CONDITION x TIME ($P = 0.78$) interactions detected following the intervention (Table 1). Furthermore, there were no TIME ($P > 0.05$) or TIME x CONDITION ($P > 0.05$) interactions detected between genotypes following the intervention.

Cortical voluntary activation

Figure 5 shows the 3 levels of wrist flexor force which the subject produced in a typical trial. TMS was delivered over the left M1 during the plateau of each contraction to evoke a superimposed twitch shown in Figure 5B. As expected, the amplitude of the evoked twitches was greatest during the 50% MVIC and smallest during 100% MVIC. Figure 6 shows the change in cortical voluntary activation following 4 consecutive sessions of sham and anodal tDCS. Voluntary activation levels were similar between sham and anodal tDCS conditions at baseline ($P > 0.05$). Following the intervention there was a main effect for TIME ($P = 0.0015$) and a CONDITION x TIME interaction ($P = 0.0003$). *Post hoc* analysis showed that following 4 sessions of anodal tDCS, cortical voluntary activation increased from 88.14 ± 1.60% to 91.33 ± 1.24% compared to sham tDCS (88.54 ± 1.57% to 87.48 ± 1.85%). There were no TIME ($P > 0.05$) or TIME x CONDITION ($P > 0.05$) interactions detected between genotypes following the intervention.

DISCUSSION

We investigated the cumulative effect of 4 consecutive bouts of anodal tDCS on muscle strength and indices of M1 plasticity, in particular cortical voluntary activation and the influence of the *BDNF* polymorphism on these responses. The main finding of this study was that repeated sessions of anodal tDCS increased cortical voluntary activation and isometric wrist flexor strength compared to repeated sessions of sham tDCS. Corticospinal excitability increased and silent period duration decreased following anodal tDCS, demonstrating the induction of M1 plasticity. Although we found no significant genotype by time interactions, the within main effects for increased corticospinal excitability and decreased silent period duration in the *Val/Val* individuals following anodal tDCS, is an important new finding that warrants some discussion.

Repeated sessions of anodal tDCS increased isometric strength and cortical voluntary activation

To date, only a limited number of studies have examined the cumulative effect of anodal tDCS on motor performance.[11, 12] The increase in isometric wrist muscle strength is similar to other studies that have reported an improvement in fine motor control of the hand following repeated sessions of tDCS (anodal and cathodal), [11, 12] but, we report increased cortical activation, which is a new finding.

Although the mechanisms of force gradation are well-described, [36] it has not been established whether improved force production following tDCS is associated with increased cortical voluntary activation or M1 plasticity. To this end, as cortical voluntary activation is a measure of the level of neural drive to a muscle and reflects motor cortical drive, the finding of increased cortical voluntary activation following anodal tDCS illustrates that NIBS increases the net motor output (i.e. neural drive) from the M1 to the wrist flexors only. Therefore, accumulated bouts of anodal tDCS improves voluntary drive at the level of the M1, which presents as an increase in wrist flexor muscle strength. The improvement in cortical voluntary activation is likely a result of tDCS modulating synaptic efficacy which has improved the net descending drive (i.e. increased motor cortical drive) to the motoneuron pool.

It is unclear why anodal tDCS had no effect on wrist extensor strength or M1 plasticity. Although it is well established that the M1 can undergo both rapid, reversible, and long-term plastic changes, and that shifts in body representations provide an insight into how various body parts can reorganize relative to one another[37], such plastic changes do not inform us whether all muscles in a given body part reorganize in a similar manner and to the same extent. Based on the current findings, tDCS over the wrist flexor region had no effect

on muscle strength or indices of plasticity of the wrist extensor. These findings show that the wrist flexors differ in their potential to undergo plasticity following anodal tDCS compared to the wrist extensors, despite how anatomically close these muscles are on M1.[38, 39]

Corticospinal excitability and inhibition following accumulated bouts of anodal tDCS in different BDNF genotypes

The finding that corticospinal excitability increased following multiple sessions of anodal tDCS is consistent with the results from a previous study which also reported increased MEP amplitudes following 5 consecutive sessions of anodal tDCS.[40] However, our study extends these findings by demonstrating that anodal tDCS produces general enhancement of corticospinal excitability, by changing the gain in the stimulus-response curve. The increase in MEP amplitude of the target muscle following anodal tDCS reflects elements of M1 plasticity via mechanisms associated with long-term potentiation (LTP).[41,42] The mechanisms mediating the after-effects of tDCS are well described, and the general consensus is that the after-effects are associated with a change in synaptic strength due to modulation of the NMDA receptor.[6, 43] Involvement of the NMDA receptor is highlighted by pharmacological studies in which the after-effects of anodal tDCS are supressed following the use of the NMDA-receptor antagonist, dextromethorphan.[41] The increased MEP amplitude evoked by TMS in this experiment provides evidence that cumulative bouts of anodal tDCS have specifically modulated corticospinal connections (i.e. improved synaptic efficacy) that potentially favor the production of force and are likely reinforced as a result of mechanism associated with LTP.

The role of the *BDNF* polymorphism in modulating M1 plasticity in humans is less established compared to animal models, however, the findings of this study are consistent with previous studies that have shown that M1 plasticity is differentially modulated following

experimentally-induced plasticity.[44] For example, it has been reported that there is an 18% to 30% reduction in activity-dependent secretion of *BDNF* in *Val/Met* allele carriers.[19] In our study, the *BDNF* polymorphism shaped the induction of M1 plasticity following repeated sessions of anodal tDCS. The significant increase in MEP amplitude observed following anodal tDCS was a result of *Val/Val* and *Val/Met* individuals being pooled. However, when the pooled individuals were sub-grouped into genotype, and individual data were examined, the *Val/Val* individuals showed a greater increase in MEP amplitude compared to *Val/Met* individuals. However, due to the small sample size of the *Val/Met* group, this magnitude was not statistically significant. Although there were no genotype interactions, the within-time effects warrant some discussion. For example, at 150% AMT, the mean MEP amplitude in *Val/Val* individuals increased by 55% compared to a 4% increase in those with the *Val/Met* polymorphism. It is likely that the significant increase in corticospinal excitability we observed is a product of the larger representative sample of *Val/Val* individuals. However, in contrast to these experimental findings, it has been found that carriers of the *BDNF Met* allele (*Val/Met*) display enhanced corticospinal responses to both anodal and cathodal tDCS protocols compared to the *Val/Val* genotype.[20, 45] The differences may lie in the experimental methodology, as a single session of anodal tDCS may only be sufficient to modify the transmembrane neuronal potential.[20, 45]

TMS and neuroimaging studies have reported a profound effect of the *BDNF* polymorphism on cortical morphology [46] and synaptic activity (LTP, efficacy of neural transmission, brain activation volumes) [47 -49] underlying plasticity. Based upon this, the time effect we found for inducing M1 plasticity in only the *Val/Val* participants following anodal tDCS, supports the important role that the *BDNF* polymorphism plays in shaping experimentally–induced M1 plasticity.

Understanding the effects of anodal tDCS on intracortical inhibition is important, as modulation of SICI is crucial for motor performance. Interestingly, we observed no changes in SICI following 4 consecutive sessions of anodal tDCS. Although this was an unexpected finding, this suggests that accumulated bouts of anodal tDCS appear to modulate $GABA_B$ rather than $GABA_A$ neurons; however, it is unclear as to why. However, we did report a reduction in silent period duration. Since the silent period that follows the excitatory MEP is caused by activation of long-lasting $GABA_B$ mediated inhibition and reflects a temporary suppression in motor cortical output[50], it appears that cumulative bouts of anodal tDCS specifically target neural circuits that use $GABA_B$ as their neurotransmitter, resulting in the release of pyramidal tract neurons from inhibition. Therefore, a reduction in the temporary suppression of motor cortical output may be a putative neural mechanism underlying the changes in cortical voluntary activation.

Interestingly, the *BDNF* polymorphism did not differentially affect muscle strength or cortical voluntary activation. Instead, the *BDNF* polymorphism appears to influence indices of M1 plasticity. Given the small sample size and lack of representation from *Met/Met* individuals, this is speculative. Indeed, a larger sample size of each *BDNF* genotype would allow for correlational analyses of changes in neurophysiological parameters and genotype, which would further strengthen the potential influence of the *BDNF* polymorphism on motor function and plasticity. However, these preliminary data highlight the importance of investigating the role of the *BDNF* polymorphism in induction of M1 plasticity and whether this may manifest as a difference in motor performance. Another limitation to this study is that measures at a segmental level, particularly cervicomedullary MEPs and H-reflex were not recorded, which would have provide additional information as to the site of adaptation within the corticospinal tract following stimulation.

Collectively, these findings show that repeated session of anodal tDCS induced M1 plasticity and increased cortical voluntary activation which manifests itself as an improvement in isometric muscle strength. The induction of M1 plasticity appears to be influenced by the *BDNF* polymorphism, however these data should be interpreted with caution given the limited sample size and warrant further investigation.

At a minimum, the clinical implications for these findings suggest that accumulated bouts of anodal tDCS could be used in participants that have deficiencies in muscle strength, as the *BDNF* polymorphism, only appears to affect the induction of plasticity and not strength development.

ABBREVIATIONS

AMT: active motor threshold

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

ECR: extensor carpi radialis

FCR: flexor carpi radialis

LTD: long-term depression

LTP: long-term potentiation

MEPs: Motor-evoked potentials

MVIC: maximal voluntary isometric contraction

MEP: motor evoked potentials

M1: primary motor cortex

NIBS: non-invasive brain stimulation

rmsEMG: root-mean squared electromyography

SICI: short-interval intracortical inhibition

sEMG: surface electromyography

tDCS: transcranial direct current stimulation

TMS: transcranial magnetic stimulation

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Table 1: Mean **(**± SE) for M_{MAX}, single-pulse TMS pre-stimulus rmsEMG at 130% AMT, paired-pulse TMS pre-stimulus at 80% and 120% AMT (CS, TS respectively) and SICI prior to and following 4 consecutive sessions of sham and anodal tDCS.

	Sham tDCS		**Anodal tDCS**		
	Pre	Post	Pre	Post	***P* value**
M_{MAX} (mV)	7.21 ± 0.69	7.32 ± 0.61	7.63 ± 0.82	7.25 ± 0.78	> 0.9999
SP rmsEMG (% $rmsEMG_{MAX}$)	1.01 ± 0.40	1.12 ± 0.40	0.83 ± 0.16	0.90 ± 0.15	0.1191
PP rmsEMG (% $rmsEMG_{MAX}$)	0.86 ± 0.23	0.91 ± 0.18	0.63 ± 0.10	0.82 ± 0.12	0.6960
SICI	36.64 ± 4.93	38.06 ± 6.45	42.03 ± 6.06	38.06 ± 6.60	> 0.9999

M_{MAX}: maximum compound wave; SICI: short-interval intracortical inhibition; SP: single-pulse; PP: paired-pulse; TMS: transcranial magnetic stimulation.

Table 2: Mean **(**± SE) for MEP amplitudes expressed at percentage of M_{MAX} at 110-210% AMT (increments of 20%) prior to and following 4 consecutive sessions of sham and anodal tDCS.

MEP Amplitude (%M_{MAX})	**Sham tDCS**		**Anodal tDCS**		
	Pre	Post	Pre	Post	**P value**
110% AMT	6.33 ± 0.84	6.42 ± 0.85	6.60 ± 0.97	11.04 ± 1.66*†	0.0004
130% AMT	12.64 ± 1.69	13.60 ± 2.03	11.70 ± 1.75	19.29 ± 2.56*†	0.0104
150% AMT	17.03 ± 2.20	17.14 ± 2.06	15.54 ± 2.25	23.53 ± 3.45*†	0.0332
170% AMT	18.91 ± 2.45	19.87 ± 2.70	18.83 ± 2.47	28.41 ± 4.35*†	0.0040
190% AMT	21.52 ± 2.59	21.45 ± 1.77	21.67 ± 2.79	28.74 ± 4.68*†	0.0162
210% AMT	24.22 ± 3.46	21.92 ± 1.94	21.55 ± 2.65	29.66 ± 4.95*†	0.0323

* significant to sham tDCS condition ($P > 0.05$); † significant to baseline ($P > 0.05$).

FIGURE LEGENDS

Figure 1: Schematic representation of the experimental design with measures obtained prior to and following 4 consecutive sessions of sham and anodal tDCS. Pre- and post-measures included assessment of peripheral muscle excitability (M-waves), corticospinal excitability and inhibition recruitment curves, cortical voluntary activation, short-interval intracortical inhibition (SICI), and maximal voluntary isometric contraction (MVIC) strength test of the right wrist flexors and extensors.

Figure 2: Mean (± SE) changes in MVIC strength of the right wrist flexors following 4 consecutive sessions of sham and anodal tDCS. * significant to sham tDCS; † significant to baseline. Anodal tDCS stimulation resulted in an 8% increase in isometric wrist flexor strength compared to 3% following sham tDCS.

Figure 3: Mean (± SE) changes in MEP amplitude following 4 consecutive sessions of **(A)** sham tDCS and **(B)** anodal tDCS. **(C)** changes in MEP amplitude before and after 4 consecutive sessions of anodal tDCS in healthy subjects with different *BDNF* genotypes. *significant to sham tDCS; †significant to baseline.

Figure 4: Mean (± SE) changes in silent period duration following 4 consecutive sessions of **(A)** sham tDCS and **(B)** anodal tDCS. **(C)** changes in MEP amplitude before and after 4 consecutive sessions of anodal tDCS in healthy subjects with different *BDNF* genotypes. *significant to sham tDCS; †significant to baseline.

Figure 5A-C: (A) Raw force traces for 3 levels of wrist flexor voluntary contraction force taken from a representative subject in a typical testing trial. TMS was delivered over the contralateral motor cortex during 100%, 75%, and 50% MVIC. **(B)** Raw traces of the superimposed twitches produced by cortical stimulation during 100%, 75%, and 50% MVIC. **(C)** Raw EMG responses (MEPs) produced by cortical stimulation during 100%, 75%, and 50% MVIC.

Figure 6: Mean (± SE) changes in cortical voluntary activation following 4 consecutive sessions of sham and anodal tDCS. * significant to sham tDCS; † significant to baseline.