A dissociation between propriospinal facilitation and inhibition after bilateral transcranial direct current stimulation

Alana B. McCambridge,1,2 James W. Stinear,1,2 Winston D. Byblow1,2

1 Movement Neuroscience Laboratory, The University of Auckland, Auckland, New Zealand.
2 Centre for Brain Research, The University of Auckland, Auckland, New Zealand.

Running head:

   tDCS effects on propriospinal neurons

Corresponding author:

Winston, D. Byblow
Movement Neuroscience Laboratory
Centre for Brain Research
The University of Auckland
Auckland, New Zealand
Phone: 373 7599 x 84897
Fax: 373 7043
Email: w.byblow@auckland.ac.nz
Abstract

Propriospinal premotoneurons (PN) are essential for accurate control of the upper limb. They receive bilateral input from premotor (PM) and primary motor (M1) cortices. In humans, excitability of PNs can be estimated from motor evoked potentials (MEPs) by pairing a descending volley using transcranial magnetic stimulation (TMS) to summate with an ascending volley from peripheral nerve stimulation at the C3-C4 level of the spinal cord. Transcranial direct current stimulation (tDCS) alters excitability of cortical and subcortical areas. A recent study demonstrated that cathodal-tDCS can suppress facilitatory (FAC) and inhibitory (INH) components of PN excitability, presumably via effects on cortico-reticulospinal neurons (Bradnam et al., 2011). The present study investigated the effects of bilateral tDCS with healthy subjects. The cathode was placed over left dorsal PM or M1, and the anode over right M1 in separate sessions (PM-M1, M1-M1 or Sham). TMS of right M1 elicited MEPs in left Biceps Brachii across a range of TMS intensities chosen to examine PN-mediated FAC and INH. Conditioning was applied using median nerve stimulation with an interstimulus interval that coincided with TMS and peripheral volleys summing at the C3-C4 level. All participants showed FAC at TMS intensities near active motor threshold and INH at slightly higher intensities. After tDCS, FAC was reduced for M1-M1 compared to Sham but not after PM-M1 stimulation. Contrary to an earlier study with cathodal-tDCS, INH was unchanged across all sessions. The difference between these and earlier findings may relate to dual vs single hemisphere M1 stimulation. M1-M1 tDCS may be a useful adjuvant to techniques that aim to reduce upper limb impairment after stroke.

Key words: propriospinal, direct current stimulation, motor cortex, transcranial magnetic stimulation, electrode montage
Introduction

Propriospinal premotoneurons (PN) at the level of the cervical 3rd and 4th segments (C3-C4) of the spinal cord are important for accurate control of the upper limb. Studies in cat and macaque have demonstrated the need for PNs during goal directed movements through permanent transection of the spinal cord (Sasaki et al., 2004, Alstermark et al., 2011). In the macaque Kinoshita et al. (2012) temporarily blocked synaptic transmission of C3-C4 PNs while sparing other motoneuron inputs. This impaired the monkeys’ ability to accurately reach and grasp. By using a reversible blockade Kinoshita et al. (2012) confirm C3-C4 PNs are critical in an intact nervous system for producing accurate upper limb movement.

Animal C3-C4 PNs receive input from contralateral and ipsilateral primary motor (M1) and premotor (PM) areas (Andrews et al., 1973, Catsman-Berrevoets and Kuypers, 1976). Contralateral input from the crossed corticospinal tract and ipsilateral input from the reticulospinal tract (RST) converge onto alpha motoneurons (α-MN), PNs, and spinal inhibitory interneurons (IN) rostral to α-MNs in the spinal cord (Brinkman and Kuypers, 1973, Catsman-Berrevoets and Kuypers, 1976, Illert et al., 1977, Illert et al., 1981, Boudrias et al., 2010). In human and non-human primates, descending inputs to spinal INs tonically inhibit C3-C4 PNs (Alstermark et al., 1999, Nicolas et al., 2001, Pierrot-Deseilligny and Burke, 2005). This inhibition is released to produce goal-directed movements that require coordination of proximal and distal muscles (Pierrot-Deseilligny, 1996, Pierrot-Deseilligny and Burke, 2005, Isa et al., 2006, Alstermark et al., 2007, Giboin et al., 2012). Divergent projections of a single PN onto proximal and distal α-MNs have been found in the cat (Alstermark et al., 1990). Such divergence in humans is presumed to assist multi-joint coordination of the upper limb (Pierrot-Deseilligny, 1996, Pierrot-Deseilligny and Burke, 2005).
The C3-C4 propriospinal system can be investigated in humans by pairing peripheral nerve stimulation with transcranial magnetic stimulation (TMS) at an interstimulus interval (ISI) that allows summation of afferent and efferent volleys at C3-C4 PNs (Figure 1) (Nicolas et al., 2001, Stinear and Byblow, 2004a, b, Bradnam et al., 2011). This technique can be used to evoke facilitation (FAC) and inhibition (INH) components of presumed propriospinal function. Peripheral nerve stimulation facilitates TMS-induced motor evoked potentials (MEP) when combined with low intensity TMS. Facilitation of the MEP occurs as both volleys summate onto PNs (Nicolas et al., 2001, Iglesias et al., 2007, Roberts et al., 2008). At a slightly higher TMS intensity the MEP is inhibited (Nicolas et al., 2001). A stronger TMS intensity presumably stimulates higher threshold neurons that preferentially innervate INs rostral to PNs (Nicolas et al., 2001, Iglesias et al., 2007, Roberts et al., 2008, Bradnam et al., 2011). Using this technique the components of propriospinal excitability have been shown to be dis-inhibited after a stroke (Stinear and Byblow, 2004a), and suppressed by ipsilateral M1 cathodal transcranial direct current stimulation (tDCS) in healthy participants (Bradnam et al., 2011).

tDCS is a non-invasive brain stimulation technique that can modulate cortical (Lang et al., 2011) and subcortical excitability (Lang et al., 2005, Bolzoni et al., 2013b) by inducing a weak direct current to the brain via electrodes placed over the scalp. The conventional unilateral montage positions an ‘active’ electrode over the target area (e.g. M1) and ‘reference’ electrode over contralateral supraorbit. An alternate electrode montage is dual hemisphere or bilateral tDCS. This montage positions the anode and cathode over opposite M1 thereby stimulating both M1 simultaneously (Mordillo-Mateos et al., 2012, Sehm et al., 2013). Placement of anode or cathode over M1 respectively facilitates or suppresses MEP amplitude in the contralateral arm (Paulus, 2011).
Neuromodulation of C3-C4 propriospinal excitability may be important after stroke for redressing abnormal propriospinal excitability in the affected arm (Mazevet et al., 2003, Stinear and Byblow, 2004a). Recently unilateral tDCS was shown to modulate excitability of descending pathways controlling the ipsilateral proximal upper limb (Bradnam et al., 2011, McCambridge et al., 2011, Bradnam et al., 2012, O'Shea et al., 2014). Bradnam et al. (2011) found that unilateral M1 cathodal tDCS (c-tDCS) suppressed both FAC and INH components of propriospinal excitability to the ipsilateral arm of healthy participants. The present study aimed to investigate the effect of bilateral tDCS on crossed and uncrossed descending pathways affecting PN excitability in healthy participants. Since C3-C4 PNs are bilaterally innervated from both PM and M1 (Nathan et al., 1996, Yeo et al., 2012), we investigated placement of the cathode over ipsilateral PM or M1 in separate sessions (M1-M1, PM-M1, Sham). We expected that FAC and INH would again be suppressed for the arm ipsilateral to the cathode, as previously shown (Bradnam et al 2011, 2012; McCambridge et al 2011). However, with bilateral tDCS we expected that the anode over the contralateral M1 might counteract suppressive effects of the cathodal stimulation on spinal inhibitory interneurons, thereby minimising the effects on PN-mediated INH. We also expected suppression of ipsilateral motor evoked potentials (iMEP) after real tDCS indicative of tDCS effects on uncrossed descending pathways (Bradnam et al 2011). Given the complexity of the protocol to obtain propriospinal and ipsilateral-mediated MEPs, measures of interhemispheric inhibition could not also be made within the timeframe of expected post-tDCS effects, and were not studied. Neurophysiological (Sehm et al., 2013, O'Shea et al., 2014), behavioural (Vines et al., 2008, Kang and Paik, 2011, Mahmoudi et al., 2011) and computer modelling (Miranda et al., 2006, Datta et al., 2011) studies have highlighted the differential effects of unilateral versus bilateral tDCS. Understanding bilateral tDCS effects on PN excitability may be an important step toward exploring future clinical applications for tDCS.
Methods

Participants

Based on sample-size calculations determined from Bradnam et al. (2011; alpha error probability = 0.05; power = 0.95), twelve healthy adults (mean age 28.3 yrs, range 21 - 48 yrs, 5 males) without history of upper limb injury or neurological disorder participated in the study. Eleven participants were right handed, and one participant was ambidexterous, as assessed with the Edinburgh Handedness Inventory (Oldfield, 1971). All participants were screened for contraindications to TMS by a neurologist, and provided informed consent in accordance with the declaration of Helsinki. The study was approved by the local ethics committee.

Task positions

To examine PN excitability participants were seated with their left elbow resting on the chair arm rest while holding a 450 g weight in their left hand. The left forearm was supinated, wrist neutral, and fingers loosely gripped the weight. The left hand was held above a surface to maintain a consistent elbow angle of approximately 90 degrees flexion (45 degrees from horizontal). The right arm remained at rest. This task and posture has been successfully used by others (Bradnam et al., 2011) to facilitate summation of peripheral nerve stimulation and TMS at C3-C4 PNs. To evoke iMEPs, both elbows were fixed at 90 degrees flexion, forearms supinated and wrists strapped to provide resistance. Figure 2 B-D provide a schematic of the task and subject posture.

Electromyography

Surface electromyography (EMG) was recorded from left and right biceps brachii (BB), left flexor carpi radialis (FCR) and right FDI muscles via disposable electrodes (30 mm
x 20 mm; Ambu, Ballerup, Denmark). EMG was recorded from all muscles in a belly-tendon montage. EMG signals were amplified (CED 1902; Cambridge Electronic Design (CED), Cambridge, United Kingdom), band-pass filtered (2 – 1000 Hz), and sampled at 2 kHz (CED 1401).

Transcranial magnetic stimulation

Single-pulse TMS was delivered to left and right M1 with a figure-of-8 D70² coil and Magstim 200 unit (Magstim, Dyfed, United Kingdom). The coil was held tangentially to the scalp and positioned to induce current posterior to anterior across the central sulcus. The optimal site for eliciting contralateral MEPs in the left BB was marked on the scalp over the right hemisphere. The optimal site for eliciting MEPs in the right BB was marked on the scalp over the left hemisphere, and used as the ‘hotspot’ for left BB iMEPs. The optimal site for eliciting MEPs in the right FDI was similarly marked over the scalp over the left hemisphere and used to collect MEPs in the right FDI.

In right M1, active motor threshold (AMT) was defined for left BB as the minimum stimulus intensity that elicited a 200 µV MEP in four out of eight trials while maintaining task position. Six stimulus intensities were used in this task starting from AMT – 2% maximum stimulator output (MSO) and increasing in 2% MSO steps to AMT + 8% MSO. The order of intensities was block randomised and each block consisted of 12 non-conditioned MEPs (NC) and 12 MEPs conditioned with median nerve stimulation (C) in a random order. Rest breaks were taken between blocks to prevent fatigue.

To evoke iMEPs in the left BB, participants performed an isometric bilateral elbow flexion contraction of approximately 30 – 40% of their maximum. Twelve 100% MSO stimuli were delivered at a rate of 0.2 Hz over the left M1 with rest periods provided to avoid
fatigue. Ear plugs were worn by the participants for protection with high stimulation intensities.

Contralateral MEPs were also obtained from right FDI at rest pre, immediately post (Post0) and 30 minutes post tDCS (Post30). The TMS intensity was set to produce stable 1 – 1.5 mV MEPs at baseline.

**Median nerve stimulation**

A Digitimer DS7A constant current stimulator (Digitimer, Hertfordshire, United Kingdom) delivered 1 ms square wave pulse to the median nerve of the left arm. The custom made stimulating clamp comprised of a metal ball (9.5 mm diameter) as the cathode and a flat square (7.3 cm²) metal surface as the anode. The clamp was placed slightly proximal to the medial and lateral epicondyle of the humerus. The cathode was on the medial aspect and the anode on the lateral aspect of the elbow. The median nerve motor threshold was defined as the minimum current intensity to induce a response (M wave) of 0.1 mV in FCR while maintaining task position. The median nerve stimulation intensity was set at 0.8 x motor threshold to preferentially stimulate group I sensory afferents (Nicolas et al., 2001).

For conditioned trials median nerve stimulation preceded the TMS pulse to allow summation of the two stimuli at the level of C3-C4 PNs. This interstimulus interval (ISI) was based on an estimated 6 ms efferent conduction time from M1 to C5-C6 α-MNs and 10 ms afferent conduction time from median nerve stimulation to C5-C6 α-MNs. Therefore, a 4 ms ISI would produce summation at the C5-C6 BB α-MNs, with an additional 3-4 ms expected to produce summation at the C3-C4 premotoneuronal PNs (see Pierrot-Deseilligny and Burke (2005) for estimation of central conduction times). The optimal ISI was presumed to be 7-8 ms and optimised for each individual by also considering 6 ms and 9 ms if necessary (Bradnam et al., 2011). TMS intensity of AMT + 2% MSO was used to collect a randomised
block of 12 NC MEPs and 12 C MEPs at both ISI’s of 7 and 8 ms. The appropriate ISI was chosen when the ratio between the C MEP average and the NC MEP average was at least > 1.1 (Ratio = C / NC). A ratio > 1 represents facilitation, and < 1 inhibition of the MEP. If facilitation was not present at 7 or 8 ms, then a randomised block of 12 NC MEPs and 12 C MEPs at ISI’s of 6 and 9 ms was also collected. The ISI which provided the maximum facilitation was chosen as the optimal ISI and remained constant for the experiment.

**Study design**

Participants completed three experimental sessions of bilateral tDCS. The cathode was placed over left PM or M1 and anode over right M1. TDCS was either real (1 mA, 15 min) or Sham. The sessions were randomized and separated by at least 5 days. Both participants and the experimenter were blinded to stimulation type.

**Transcranial direct current stimulation**

Bilateral tDCS was delivered with a constant current of 1 mA for 15 min using a Phoresor II stimulator (Model PM850; IOMED) via conductive rubber electrodes placed in saline soaked sponges. The cathode (18 cm²) was placed over the left hemisphere, targeting either left PM or left M1 BB hotspot. The anode (42 cm²) was placed over the right M1 BB hotspot. Left PM was presumed to be located 2.5 cm anterior and 1 cm medial to the FDI hotspot as defined by Fink et al. (1997) and used in previous studies targeting PM (Rizzo et al., 2004, Koch et al., 2007) (Figure 2A). An unblinded experimenter applied real (PM-M1 or M1-M1) or Sham stimulation but took no part in data collection or analysis. For real stimulation the current was ramped up to 1 mA over 15 s and ramped down to 0 mA at the end of stimulation over 15 s. For Sham stimulation, the current was ramped up to 1 mA over 15 s and then ramped down from 1 mA to 0 mA over 30 s (Gandiga et al., 2006) with the
placement of the cathode between PM or M1 randomised amongst participants. Participants were instructed to sit quietly throughout tDCS with their eyes open.

Dependant measures

Left BB contralateral MEP (cMEP) area was calculated as the integral of the rectified EMG within a 20 ms window from cMEP onset, after subtracting baseline area from an equivalent pretrigger time window, and expressed as a ratio (C / NC) for each TMS intensity. The TMS intensity that produced maximum facilitation (FAC) was AMT or AMT +2% MSO (median = AMT + 2%MSO). The intensity that produced maximum inhibition (INH) at a TMS intensity higher than FAC was AMT + 4% or AMT + 6% MSO (median = AMT + 6%MSO) (see Figure 3 for example of one representative subject). The change in FAC and INH components after tDCS (ΔFAC, ΔINH) was calculated as Δ = post tDCS – pre tDCS.

Left BB iMEPs were identified from rectified EMG traces. An iMEP was deemed present when the EMG exceeded the mean pre-trigger EMG + 1 standard deviation for more than 5 ms within a predetermined iMEP window (18 ms to 30 ms) (Ziemann et al., 1999). The latency of left BB iMEP was measured from the individual rectified EMG traces. For each participant, the earliest onset and latest offset of the iMEP across all sessions and time points was used as their individualised iMEP window. Ipsilateral MEP area was calculated as the integral of the rectified EMG within each individual’s iMEP window, after subtracting an equivalent window of pre-trigger EMG. ΔiMEP was calculated as Post tDCS – Pre tDCS.

As a manipulation check for the expected response to tDCS, cMEPs were elicited in right FDI with peak to peak amplitude measured and averaged for each time point (pre, post 1, post 2). ΔcMEP was calculated as Post tDCS – Pre tDCS.

Statistical analyses
FAC and INH ratios at baseline were analysed with one-sample t-tests to confirm a difference from 1 in all sessions. A 2 x Component (FAC, INH) 3 x Session (M1, PM, Sham) 2 x Time (Pre, Post) rmANOVA was used to examine for effects of tDCS. ΔFAC and ΔINH were analysed with paired-sample t-tests to compare each active tDCS session with Sham, and one-sample t-tests to test for differences from 0.

To assess for other factors that may influence the ratios between sessions, a one-way ANOVA was performed on AMT (%MSO). Two related-sample binomial sign tests compared the stimulation intensity that elicited the FAC or INH component pre versus post tDCS within each session. For each component (FAC, INH) a 3 Session (M1, PM, Sham) x 2 Time (Pre, Post) rmANOVA was also performed on the NC MEP area.

Due to an unequal prevalence of iMEPs between sessions, paired-sample t-tests were used to compare iMEP latency and area pre and post tDCS, and ΔiMEP was analysed using a one-sample t-test.

A 3 Session (M1, PM, Sham) x 3 Time (Pre, Post0, Post30) rmANOVA was used to investigate changes in right FDI MEP amplitude, and ΔcMEP analysed using a one-sample t-test.

For all tasks, root mean square EMG (EMGrms) was calculated for a 90 ms window before stimulus onset to estimate any effect of contraction strength on MEP data. The left BB EMGrms from the propriospinal task was assessed with a 3 x Session (M1, PM, Sham) 2 x Condition (NC, C) 2 x Time (Pre, Post) 6 x Intensity (-2%, AMT, +2%, +4%, +6%, +8%) rmANOVA. The left BB EMGrms from the iMEP task was analysed using a 3 x Session (M1, PM, Sham) 2 x Time (Pre, Post) rmANOVA. Right FDI EMGrms was analysed using a 3 x Session (M1, PM, Sham), 3 x Time (Pre, Post0, Post30) rmANOVA.
Effects were deemed significant if \( p < 0.05 \). Means ± standard error (S.E) are reported in the text.

**Results**

**Facilitation and inhibition of left BB MEPs**

Prior to tDCS, FAC and INH were confirmed and differed from 1 in all sessions (all \( p < 0.002 \)) (Figure 4A). The rmANOVA revealed a main effect of component (\( F_{1,11} = 147.15, p < 0.001 \)) with FAC > INH, and no other effects. Analysis of the FAC and INH components separately revealed no main effects or interactions (Facilitation: Session x Time interaction \( F_{2,22} = 2.771, p = 0.085 \); Inhibition: Session x Time interaction \( F_{2,22} = 0.032 \ p = 0.969 \)).

Paired t-tests of \( \Delta \)FAC showed that M1-M1 stimulation -0.32 ± 0.16 produced less facilitation than Sham 0.15 ± 0.17 (\( t_{11} = -2.34, p = 0.039 \)) but PM-M1 stimulation 0.05 ± 0.13 did not differ from Sham (\( t_{11} = 0.44, p = 0.67 \)) or M1-M1 stimulation (\( t_{11} = 1.84, p = 0.094 \)) (Figure 4B). One sample t-tests confirmed that \( \Delta \)FAC was significantly reduced relative to baseline after M1-M1 tDCS (\( t_{11} = -2.537, p = 0.028 \)) with no other tDCS effects on \( \Delta \)FAC or \( \Delta \)INH in any session (all \( p > 0.5 \)). There were no effects of tDCS on \( \Delta \)INH (M1-M1 0.017 ± 0.035, PM-M1 0.032 ± 0.047, Sham 0.022 ± 0.045; all \( p > 0.79 \)).

AMT did not differ between sessions (M1-M1 35 %MSO ± 1.6, PM-M1 34.7 %MSO ±1.3, Sham 35 %MSO ±1.6; \( F_{2,33} = 0.028, p = 0.972 \)). The TMS intensity that elicited FAC (AMT + 1.4%MSO ± 0.24) and INH (AMT + 5.47%MSO ± 0.22) was not changed by tDCS (all \( p > 0.109 \)). The NC MEP area was not changed by tDCS in either session, for either FAC or INH components (all \( p > 0.084 \)).

**Left BB iMEPs**
iMEPs were present in all 3 sessions for 7/12 participants, and in 2 sessions only for 3/12 participants. iMEP area showed no significant differences pre to post tDCS (all $p > 0.54$). $\Delta i$MEP was not significantly different from 0 (all $p > 0.54$). iMEP latency was unchanged pre to post tDCS (all $p > 0.15$) (M1 pre = 19.04 ± 0.77 ms, post = 20.06 ± 0.58 ms; PM pre = 19.77 ± 0.29 ms, post = 19.8 ± 0.60 ms; Sham pre = 20.99 ± 0.56 ms, post = 20.35 ± 0.65 ms).

**Right FDI**

There were no main effects or interactions for FDI MEP amplitude (all $p > 0.18$). One-sample t-tests of post 1 and post 2 deltas revealed no significant differences from baseline (all $p > 0.125$).

**Pre-trigger EMG**

For all muscles and tasks analysed there were no main effects or interactions for pre-trigger EMG (M1 iMEP pre = 0.18 ± 0.03 mV vs post = 0.24 ± 0.05 mV, $p = 0.079$; all others $p > 0.195$).

**Discussion**

In the present study median nerve conditioned TMS was used to examine the effects of tDCS on spinally-mediated excitability in the upper limb. We found several confirmatory and novel results. First, median nerve conditioning facilitated left BB MEPs when combined with a low TMS intensity and inhibited left BB MEPs with a slightly higher TMS intensity. This was consistent with previous studies examining propriospinal modulation of upper limb $\alpha$-MNs (Nicolas et al., 2001, Stinear and Byblow, 2004a, b, Bradnam et al., 2011). Second, tDCS had effects on spinal networks controlling the upper limb ipsilateral to the cathode (Bradnam et al., 2011, McCambridge et al., 2011). The primary novel finding was that M1-
M1 tDCS compared to Sham suppressed PN-mediated facilitation (FAC) without affecting inhibition (INH) to the ipsilateral upper limb (BB). To our knowledge this is the first indication that tDCS may differentially modulate FAC and INH. Surprisingly, there were no effects of PM-M1 tDCS on propriospinal excitability. Potential mechanisms underlying these findings are discussed.

Previously M1 c-tDCS was shown to suppress both FAC and INH components acting on ipsilateral BB α-MNs (Bradnam et al., 2011). Here we found that M1-M1 tDCS had suppressive effects over the FAC component only. Both studies positioned the cathode over ipsilateral left M1, and used current amplitude of 1 mA for 15 minutes. Changes in corticomotor excitability after M1 tDCS have been proposed to occur via polarity dependent shifts in the resting membrane potential, whereby stimulation from the anode facilitates and cathode suppresses M1 (Nitsche and Paulus, 2000, 2001, Nitsche et al., 2005, Stagg and Nitsche, 2011). In line with this Bradnam et al. (2011) proposed FAC and INH were suppressed after M1 c-tDCS due to a general suppressive effect over ipsilateral M1 that in turn decreased excitability of ipsilateral cortico-reticulospinal input to PNs and INs in the ipsilateral BB. Therefore the differential modulation of PN components in the present bilateral montage study compared to the previous unilateral montage study seems likely to be due, at least in part, to the anode over contralateral right M1 during bilateral M1-M1 tDCS. However the site of action of anodal stimulation within the bilateral montage is unlikely to be cortical in origin. If right M1 had been facilitated after cathodal-anodal M1-M1 tDCS we would expect left BB NC MEP area to increase and/or the TMS intensity that elicited FAC or INH to decrease. This was not observed in the present study, or previously after unilateral left M1 c-tDCS (Bradnam et al., 2011). Given that M1-M1 tDCS did not change NC MEP area we propose the effects of tDCS on propriospinal excitability may, at least in part, be occurring at a subcortical level. That perhaps involves modulation of bulbo-spinal descending
projections that are known to have dense connections with PNs in the cat (Illert et al., 1978, Illert et al., 1981, Alstermark et al., 1984b). Our data supports a growing body of evidence that tDCS is capable of influencing excitability of subcortical and spinal structures (Roche et al., 2009, 2012, Bolzoni et al., 2013a, Bolzoni et al., 2013b, Bradnam et al., 2013).

Neuroimaging, animal, and computer modelling studies have illustrated the vast effects of tDCS throughout the brain (Datta et al., 2012, Bolzoni et al., 2013b, Lindenberg et al., 2013, Sehm et al., 2013). Including effects on subcortical structures such as the RST, as observed directly in the cat after anodal stimulation (Bolzoni et al., 2013b), and the midbrain and brainstem during bilateral tDCS in humans (Sehm et al., 2013). Functional connectivity measured during and after tDCS using resting state functional magnetic resonance imaging demonstrates the effects of tDCS are network wide and dynamic over time (Sehm et al., 2013). The differential effects of bilateral and unilateral tDCS may relate to the direction of current passed through the brain. Bilateral tDCS induces current directed medial to lateral in the brain, whereas unilateral tDCS induces current dorsal to ventral. In hippocampal slice preparations the current direction relative to axonal or dendritic orientation is important for determining the direction and degree of neural excitability changes (Kabakov et al., 2012). The direction of current through the whole brain may preferentially modulate different motor areas or disproportionately modulate the same areas. It is possible that cortical and subcortical neural populations interconnected with the cervical propriospinal system may be modulated differently between unilateral and bilateral tDCS leading to separate modulation of the INH component between studies. Modelling the effects of tDCS throughout the central nervous system is an important step that may begin to elucidate these matters.

Current explanations of tDCS on human propriospinal excitability are necessarily indirect and somewhat speculative. We propose that bilateral tDCS suppressed PN-mediated FAC via similar mechanisms as those proposed by Bradnam et al. (2011) after unilateral c-
tDCS, whereby cathodal stimulation of ipsilateral M1 reduced excitability of ipsilateral
cortico-reticulospinal inputs to PNs and INs. The novel finding was the preservation of INH
after M1-M1 tDCS compared to unilateral c-tDCS. This may relate to placement of the anode
over contralateral M1 within the bilateral montage. As depicted in Figure 1 PNs and INs are
innervated by ipsilateral and contralateral M1 (Andrews et al., 1973, Catsman-Berrevoets and
Kuypers, 1976). Anodal stimulation of contralateral M1 may have facilitated descending
contralateral inputs to INs and PNs, thereby counter-balancing suppression from the cathode.
Given that afferent input is stronger to INs relative to PNs, so may be the efferent input, thus
permitting a stronger effect of anodal stimulation on inhibitory INs compared to PNs (Figure
1. Thicker input to IN) (Alstermark et al., 1984a, Pierrot-Deseilligny and Burke, 2005). This
explanation is almost certainly too simplistic, but is intended as a starting point for future
hypothesis generation. The effects of bilateral tDCS may be more complicated than simply
up-regulating and down-regulating descending pathways from M1, and may also involve
bihemispheric interactions that were not measured (Lindenberg et al., 2013). M1-M1 tDCS
may have increased inter-hemispheric inhibition from right M1 to left M1 thereby supressing
the ipsilateral cortico-reticulo-propriospinal pathway leading to the observed suppression of
PN-mediated FAC. How tDCS modulates excitability at the level of the spinal cord is a topic
of ongoing investigation and requires further systematic evaluation of parameters such as
electrode montage, current density, and intersession interval for example, all of which will be
important for optimising tDCS.

PM-M1 tDCS did not alter propriospinal excitability. To our knowledge this is the
first study to examine PM-M1 bilateral tDCS. Stimulation of PM using unilateral tDCS
montages has been investigated in 3 studies with mixed results (Boros et al., 2008, Kirimoto
et al., 2009, 2011). Kirimoto et al. (2009, 2011) found PM anodal and cathodal stimulation
suppressed and facilitated contralateral MEPs respectively, but only with an electrode ≥18
cm². Whereas Boros et al. (2008) found no change after PM anodal or cathodal tDCS on MEP amplitude with single-pulse TMS. The effects of direct current stimulation targeting PM appear less robust than M1 stimulation. This may account for the lack of PM-M1 tDCS affects in the present study. In addition, we cannot rule out the possibility that cathodal-anodal PM-M1 tDCS could have had a cancelling or ceiling effect on C3-C4 propriospinal excitability. Future research should consider the use of neuro-navigation to ensure placement of electrodes is accurate for tDCS of non-primary motor areas.

There are a number of limitations to the present study. Based on previous studies, scalp measurements were used to determine placement of the cathode over PM. This method is unlikely to account for anatomical interindividual differences. Error in the placement of the electrode over PM may have increased the variability of the stimulation effect. Right FDI MEPs were collected pre and post tDCS as a manipulation check for the expected direction of modulation. Nitsche et al. (2007) found that using a small tDCS electrode targeted to adductor digiti minimi selectively modulated MEP amplitude of this muscle but not the adjacent FDI. It is possible FDI MEP amplitude was not significantly modulated after M1-M1 tDCS because the small 18 cm² cathode was targeted to right BB and not FDI hotspot. A limitation to comparing bilateral tDCS in the present study to unilateral tDCS in the previous (Bradnam et al., 2011) is the electrode sizes and thus current density were not the same (18 cm², 0.056 mA/cm²; 35 cm², 0.029 mA/cm²). A smaller cathode was used to ensure separation between PM and M1. Previous studies that manipulated current density by altering current amplitude show the extent of modulation and even the direction of change can be affected (Bastani and Jaberzadeh, 2013, Batsikadze et al., 2013). To our knowledge manipulating the size of the cathode while maintaining current amplitude during c-tDCS or M1-M1 tDCS has not yet been examined. However the possible influence of these differences should not be ignored.
Left BB iMEP area was not significantly different after M1-M1 tDCS, possibly owing to a lack of statistical power. iMEPs were successfully elicited in 7 out of 12 participants in all 3 sessions, and 3 participants in 2 sessions. Considering iMEPs are not easily found in healthy participants this was a moderate success rate in comparison to other studies (Netz et al., 1997, Bradnam et al., 2010, McCambridge et al., 2011). iMEPs are also highly variable (Ziemann et al., 1999) therefore when combined with a small sample size this makes them difficult to study. One study found c-tDCS suppressed iMEPs in the infraspinatus during a unilateral but not a bilateral contraction task that was used in this study (Bradnam et al., 2010). The infraspinatus is also more proximal than BB and perhaps receives a greater proportion of ipsilateral input relative to BB. Investigating both crossed and uncrossed descending pathways is important to further our understanding of the effects of tDCS. Modulating excitability of ipsilateral uncrossed descending pathways may be of particular importance after stroke when the contralesional ipsilateral pathway to the paretic arm becomes up-regulated (Schwerin et al., 2008, Schwerin et al., 2011, Bradnam et al., 2012, Bradnam et al., 2013).

After a stroke, many survivors experience impairment of the upper limb. Stereotypical coupling between shoulder and elbow movement, and abnormal co-contraction of upper limb muscles, can impair functional ability of the arm (Twitchell, 1951, Brunnstrom, 1970). Similar to the present study, peripheral nerve conditioned TMS has been used to examine the components of propriospinal excitability in chronic stroke patients (Stinear and Byblow, 2004a). Compared to healthy controls, FAC was increased and INH was decreased or not present in the paretic arm (Stinear and Byblow, 2004a). Due to the divergent projections of PNs, up-regulation or dis-inhibition of C3-C4 PNs make this a candidate neural system underlying abnormal muscle coupling after stroke (Mazevet et al., 2003, Stinear and Byblow, 2004a, Pierrot-Deseilligny and Burke, 2005). In particular, up-regulation of the contralesional
ipsilateral cortico-reticulo-propriospinal pathway (depicted in Figure 1) has been implicated in the expression of flexor or extensor synergies after stroke (Ellis et al., 2012, Bradnam et al., 2013). Selective suppression of FAC while maintaining INH after M1-M1 tDCS could be useful for reducing excitability of PNs. This may offer utility in promoting recovery of upper limb function. In patients with chronic stroke, M1-M1 tDCS has been shown by others to improve hand and arm function (Lindenberg et al., 2010, Bolognini et al., 2011, Mahmoudi et al., 2011, Lindenberg et al., 2012). Despite this, it is unlikely that one neuromodulation technique or tDCS montage will suit all patients. Up-regulation of propriospinal excitability in the paretic arm may be an adaptive change in some patients (Pierrot-Deseilligny, 1996, Bradnam et al., 2013). Therefore future research to distinguish markers that could identify a suitable tDCS montage for an individual patient would likely improve the efficacy of stimulation.

Acknowledgements

The authors thank Fred Noten, Sheena Sharma, and Dr James Coxon for assistance during data collection.

Grants

This work was supported by a Health Research Council PhD scholarship awarded to A. B. McCambridge.

Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

References


Figure 1. Simplified schematic of the ipsilateral and contralateral inputs to C3-C4 propriospinal system and experimental configuration. TMS over right M1 was conditioned with subthreshold median nerve stimulation at the left elbow, allowing summation of both volleys at C3-C4 PNs. All projections are excitatory except for the inhibitory interneuron and transcallosal pathway (closed circle). Input to IN may be stronger relative to PN (thicker synapse). Grey boxes indicate C3-C4 and C6-C7 level of the spinal cord. RST, reticulospinal tract; PN, propriospinal neuron; $\alpha$, alpha motoneuron; BB, biceps brachii; FCR, flexor carpi radialis.

Figure 2. Schematic diagram of the tDCS electrode positions (A) and participant task positions (B-D). A. The anode was positioned over right M1 (+) and cathode over left M1 or PM (-). PM was defined as 2.5 cm anterior and 1 cm medial to the right FDI hotspot. M1 was defined as the BB hotspot. B. Task position used to examine FAC and INH components of propriospinal excitability. LBB MEPs were conditioned with median nerve stimulation at the elbow during weak elbow flexion. C. LBB iMEPs were evoked from left M1 while the participant maintained bilateral isometric elbow flexion. D. Right FDI MEPs were collected while the participant was at rest.

Figure 3. Average rectified EMG traces of left BB MEPs from one representative subject prior to tDCS. The non-conditioned trials (grey) and conditioned trials (black) are shown at 4 TMS intensities relative to AMT. In this subject, facilitation (FAC) was at AMT + 2% MSO and inhibition (INH) at AMT + 4% MSO. The MEP area was calculated between the vertical dotted lines. The ratio of conditioned to non-conditioned (C/NC) MEP areas is indicated at the right of each trace. In the conditioned trials, median nerve stimulation (black arrow) preceded TMS (grey arrow, time 0).
Figure 4. Group averages (n = 12) of left BB MEP facilitation (black) and inhibition (grey) across all sessions (M1-M1, SHAM, PM-M1). A: Pre tDCS ratios are shown. Both facilitation and inhibition was different from 1 (horizontal dashed line) (** P < 0.002). B: Δ ratios are shown. Facilitation was suppressed after M1-M1 tDCS and Δ facilitation was different between M1-M1 and Sham sessions (* P < 0.05). Error bars indicate S.E.