Preconditioning tDCS facilitates subsequent tDCS effect on skill acquisition in older adults

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Abstract

Functional motor declines that often occur with advancing age - including reduced efficacy to learn new skills - can have a substantial impact on the quality of life. Recent studies using non-invasive brain stimulation indicate that priming the corticospinal system by lowering the threshold for the induction of long-term potentiation (LTP)-like plasticity before skill training may facilitate subsequent skill learning. Here we utilized 'priming' protocol, in which we used transcranial direct current stimulation (tDCS) applying the cathode over the primary motor cortex (M1) prior to the anode placed over M1 during unimanual isometric force control training ($FORCE_{training}$). Older individuals who received tDCS with the cathode placed over M1 prior to tDCS with the anode placed over M1 concurrent with $FORCE_{training}$ showed greater skill improvement and corticospinal excitability increases following the tDCS/$FORCE_{training}$ protocol compared to both young and older individuals who did not receive the preceding tDCS with the cathode placed over M1. The results suggested that priming tDCS protocols may be used in clinical settings to improve motor function and thus maintain the functional independence of older adults.

Key words: *healthy aging; skill acquisition;homeostatic metaplasticity; transcranial direct current stimulation; primary motor cortex*

1. Introduction

Aging is often accompanied by a decline in many domains of motor function including slowing of movements (e.g., Ketcham and Stelmach 2001), declined movement accuracy and stability (e.g., Fujiyama et al. 2013, Heuninckx et al. 2004), and reduced ability to learn new skills (e.g., Swinnen et al. 1998, Wishart and Lee 1997). It has been suggested that neurophysiological changes that occur with advancing age underpin these motor declines (Levin et al. 2014). Moreover, reduced capacity for neuroplasticity with advancing age has been observed in older adults which can contribute to behavioral impairments in the absence of significant pathology (Burke and Barnes 2006). Interestingly, despite mounting evidence indicating that older adults undergo neurophysiological changes and show a decline in motor function, the ability to acquire new skills in later life is, at least, to some extent, preserved (Voelcker-Rehage 2008).

Previous studies have shown that the functional organization of the primary motor cortex (M1) in adult mammals is constantly reshaped by behavioural demands in order to learn new motor skills (e.g., Nudo et al. 1997). This reorganisation, or neuroplasticity, is mediated, at least in part, by activity or use-dependent processes that involve synaptic modification inducing either long-term potentiation (LTP) or long-term depression (LTD) of synapses (Sanes and Donoghue 2000). As well as the brain reorganization that occurs in response to activity or use (use-dependent neuroplasticity), there is good evidence to suggest that non-invasive brain stimulation (NIBS) also induces similar neuroplastic changes in the central nervous system, at least for a short period of time (<1hr) (Nitsche et al. 2008). Transcranial direct current stimulation (tDCS) is one such form of NIBS which involves the application of a weak electrical current to the scalp, and that has been extensively used to mimic LTP- and LTD-like processes in humans (e.g., Nitsche and Paulus 2001). tDCS is thought to induce shifts in transmembrane neuronal potentials and, thus, influence corticospinal excitability (Nitsche et al. 2008). It is assumed that the neuronal changes associated with the persisting effects of tDCS are analogous to activity-dependent synaptic plasticity (i.e., LTP and LTD; Di Lazzarro et al. 2012) which is N-methyl-D-aspartate (NMDA) receptor dependent (Di Lazzaro et al. 2012). The application of tDCS over the

primary motor cortex (M1) elicits changes in corticospinal excitability in a polarity specific manner: motor evoked potentials (MEPs) evoked by transcranial magnetic stimulation (TMS) are potentiated by tDCS with the anodal electrode placed over M1and suppressed by tDCS with the cathodal electrode placed over M1 (Nitsche and Paulus 2000), suggesting the facilitatory and inhibitory nature of anodal and cathodal under the stimulation site, respectively.

Despite the abundance of research reporting tDCS effects on plasticity, in recent years large inter-individual variability in response to tDCS has been recognized (Datta et al. 2012, Fujiyama et al. 2014, Puri et al. 2015, Wiethoff et al. 2014). For example, Fujiyama et al. observed that approximately 20% of participants (8 out of 39) did not show the expected corticospinal excitability increase following tDCS with anode placed over M1. Of particular relevance is a recent paper that considered responses to tDCS in 54 healthy older (mean age = 66.9 years) (Puri et al. 2015), in which participants underwent two sessions receiving tDCS with the anode placed over M1 with different stimulation durations (i.e., 10 min and 20 min). Less than half (46%) of older adults exhibited the expected potentiation in corticospinal excitability in both sessions.

One plausible explanation for the large inter-individual variability in response to NIBS is the history of synaptic activity prior to the stimulation (Ridding and Ziemann 2010). It appears that the human motor system is regulated by homeostatic metaplasticity mechanisms (Muller et al. 2007, Murakami et al. 2012, Siebner et al. 2004). According to the Bienenstock-Cooper-Munro (BCM) theory of homeostatic metaplasticity (Bienenstock et al. 1982), plasticity at a synapse is bidirectional, resulting in either LTP or LTD. The threshold for the induction of LTP versus LTD at synapses varies according to the history of postsynaptic activity. In the presence of low previous activity of the post synaptic neuron, the synaptic modification threshold decreases, favouring the induction of LTP over LTD. In contrast, if the previous postsynaptic activity was high, the synaptic modification threshold increases which leads to the increased probability of the occurrence of LTD over LTP (Bienenstock et al. 1982). It is apparent, therefore, that the history of post-synaptic activity can affect the response to NIBS techniques.

Based on the aforementioned theory, an interesting strategy to facilitate motor skill acquisition is to decrease the threshold for induction of LTP-like synaptic plasticity by lowering neuronal activity in the M1 prior to commencing a motor training regime (Ziemann et al. 2004). Using this idea, a recent study by Christova and colleagues (2015) revealed that the application of tDCS with the cathode placed over M1 prior to tDCS with the anode placed over M1 resulted in greater improvement in motor performance (conducted simultaneously with the tDCS with the anode placed over M1) relative to the improvement in motor function observed when tDCS with the anode placed over M1 and motor training were proceeded by sham stimulation. tDCS with placing anode over M1 during skill acquisition is thought to facilitate the neuronal firing rates in task specific networks imposing additional strengthening of specific synaptic connections (Fritsch et al. 2010) and additional application of tDCS with the cathode placed over M1 prior to the tDCS with the anode placed over M1 during task acquisition is beneficial for skill acquisition by lowering the synaptic modification threshold favouring the induction of LTP. Thus, the combination of two functionally opposite tDCS protocols appeared to promote larger gains in motor performance, possibly due to homeostatic metaplasticity. However, the utilization of two mechanistically opposing tDCS protocols has never been investigated in the context of aging. There is good evidence to suggest that the responsiveness to tDCS (in terms of improving motor behaviour) is greater in older adults compared to younger adults (Hummel et al. 2010, Zimerman et al. 2013) and corticospinal excitability increases following tDCS with the anode placed over M1 are comparable between young and older adults (Fujiyama et al. 2014). As such, tDCS may have substantial potential as a clinical intervention tool to facilitate motor learning in older adults thereby potentially maintaining functional independence. In this study, we investigated the effect of tDCS with the anode placed over M1 primed with tDCS with the cathode placed over M1on motor learning and neurophysiological changes in older adults. Based on the homeostatic metaplasticity hypothesis we expected that tDCS with the cathode placed over M1 followed by tDCS with the anode placed over M1 would result in a greater skill gain and larger neurophysiological changes (e.g., greater increases in corticospinal excitability) relative to tDCS with

the anode placed over M1 in the absence of preconditioning by tDCS with the cathode placed over M1 in both young and older adults. However, we expected that the benefit of the priming protocol (down-regulation of corticospinal excitability) on subsequent skill acquisition and neurophysiological change would be limited in older adults since the ability to flexibly modulate synaptic activity declines with advancing age (Eisen et al. 1996).

2. Methods

2.1 Participants

Thirty healthy young (16 females, M = 24.8, SD = 3.0 yrs) and 30 healthy older volunteers (15 females, M = 68.0, SD = 4.6 yrs) were recruited for the study. All participants were right-handed, as assessed by the Edinburgh handedness questionnaire Oldfield 1971 (scores 85.8 ± 13.3%). Participants were screened for cognitive impairments using the Montreal Cognitive Assessment (MOCA) (Nasreddine et al. 2005), with all participants scoring within the normal range (≥ 26). Screening for contra-indications of tDCS and TMS (metal or electronic implants, chronic medical conditions, neurological conditions, substance abuse, skin irritations and pregnancy) was conducted prior to participation. A pre-experiment questionnaire revealed that no participants had any known sensorimotor or neurological deficits. The protocol was conducted in accordance with the Declaration of Helsinki (1964) and was approved by the local ethical committee of KU Leuven, Belgium. Written informed consent was obtained from all participants prior to participation. Participants were financially compensated after the study.

2.2 Experimental design

The study consisted of two sessions conducted on two consecutive days at the same time of the day. Figure 1 outlines the experimental procedure. The first session involved eight blocks of motor training (see section 2.5) with neurophysiological and behavioral assessments conducted before and after the training. The second session served as a retention test to examine behavioral performance and neurophysiological measures. In the first session, participants were randomly allocated to either a tDCS with the cathode placed over M1 followed by tDCS with the anode placed over M1 (C-A) group

(15 young, 9 females, *M* = 25.3, *SD* = 2.7 yrs; 15 older, 8 females, *M* = 68.0, *SD* = 3.2 yrs) or a sham followed by tDCS with the anode placed over M1 (S-A) group (15 young, 7 females, *M* = 25.5, *SD* = 3.3 yrs; 15 older, 7 females, *M* = 68.0, *SD* = 5.7 yrs). The C-A group received tDCS with the cathode placed over M1 for 10 min at 1.5 mA intensity prior to the 26 min of motor training during which we applied tDCS with the anode placed over M1 (20 min, 1.5 mA). The first and last blocks of the FORCE training were conducted without tDCS with the anode placed over M1, whereby tDCS with the anode placed over M1 was applied during the remaining blocks (block 2-7). The S-A group underwent an identical procedure as C-A group; the only difference was that the cathodal stimulation was replaced with sham stimulation. Motor performance and corticospinal excitability were assessed by means of $FORCE_{test}$ (see section 2.5 Isometric force control task (FORCE) section) and AURC TMS (see section 2.3 Transcranial magnetic stimulation (TMS) and electromyography (EMG) recording section), respectively. The assessment time points were before (Pre 1) and after (Pre 2) the first stimulation (cathodal for C-A group and sham for S-A group), immediately (0 min, Post 0), 20 min (Post 20), 40 min (Post 40), and 24 h (Post 24h) after cessation of last training block.

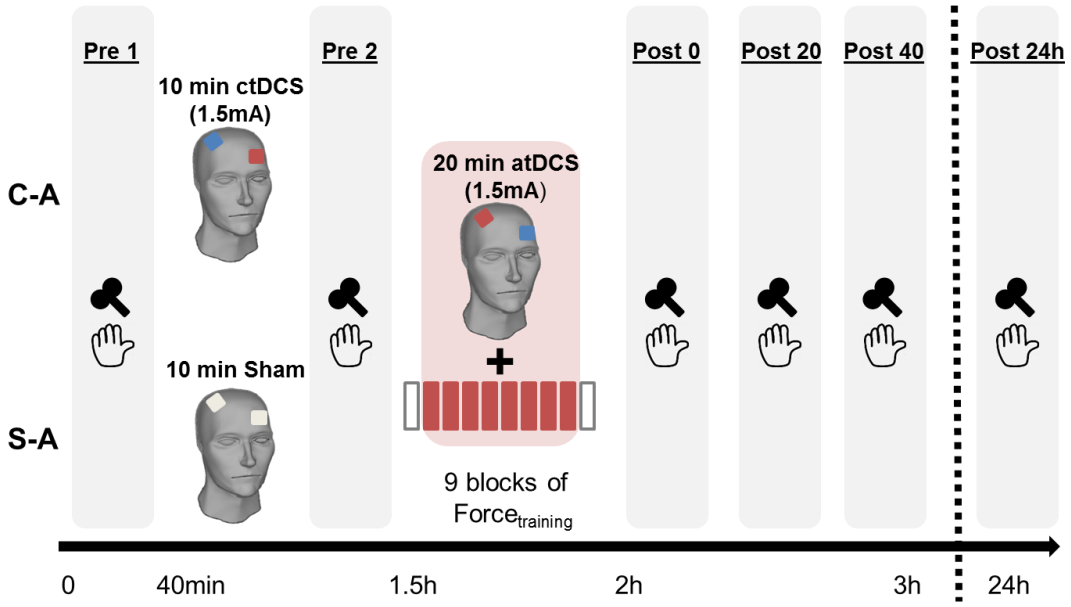

Figure 1. Schematic illustration of the experimental design. In cathodal-anodal (C-A) group, 10 min of tDCS with the cathode placed over M1 was applied prior to the application of tDCS (20min) with the anode placed over M1during FORCE training, whereas in sham-anodal (S-A) group sham stimulation REPLACED tDCS with the cathode placed over M1. Neurophysiological assessment using transcranial magnetic stimulation (TMS) and behavioral assessment ($Force_{test}$) were conducted before (Pre1) and after (Pre2) the tDCS with the cathode placed over M1 (C-A group) or sham (S-A group) and immediately (Post0), 20 min (Post20), 40 min (Post40), and 24 h (Post24h) after the cessation of FORCE training.

2.3 Transcranial magnetic stimulation (TMS) and electromyography (EMG) recording

TMS was applied to the right M1 to assess the excitability of projections from the cortical representation of the left first dorsal interosseous muscle (FDI). Single-pulse TMS was administered through a standard figure of eight coil (7 cm diameter of each wing) connected to a Magstim BiStim unit (Magstim Company, Dyfed, UK). The TMS coil was held tangentially over the scalp to induce a posterior-anterior current flow and to optimally elicit motor evoked potentials (MEPs) in the left FDI (motor hotspot). EMG surface electrodes (Ag/AgCl) were placed over the left FDI in a belly-tendon montage and signals were amplified with a gain of 1000, band pass filtered (10 – 500 Hz) and sampled at 2000 Hz using a 16-bit AD system (CED 1902, Cambridge, UK). EMG data were fed to disk for offline analysis. At the beginning of each session each individual's resting motor threshold (rMT) was determined as the lowest intensity that evoked MEPs in the FDI of greater than 50μV in at least three out of five consecutive trials (Carroll et al. 2008). To maintain constant coil positioning over the M1 hotspot of the right FDI throughout and between sessions, the position and angles of the

coil were monitored by a neuronavigation system (ANT, Enschede, The Netherlands). For navigation, a standard 3D anatomical MRI was used which was co-registered with the positions of the participant's nasion, left ear, right ear and head shape. After localization of the hotspot of the left FDI muscle in the right M1, the position was marked with a semi-permanent marker for tDCS electrode placement. With both upper limbs relaxed and resting on a pillow on the participant's lap, baseline MEP recruitment curves were constructed by applying stimuli in steps of 20% between 90% and 150% of rMT. At each intensity, 8 pulses were delivered at an inter-stimulus interval of 5 seconds (Carson et al. 2013). Accordingly, each recruitment curve took approximately 3 minutes to collect. TMS stimulation and EMG recording were controlled by Signal Software (Version 4.0, Cambridge Electronic Design, UK) and were fed to a disk for offline analysis. MEP amplitude was determined as the peak-to-peak amplitude following TMS. Trials in which root mean squared (RMS) EMG exceeded 10 μV (Carson et al. 2004) during the 40 ms immediately preceding the TMS pulse were discarded. MEPs were averaged across all trials at each time point (Pre1, Pre2, Post0, Post20, Post 40 and Post 24h) for each intensity (90%, 110%, 130%) for each participant. The mean MEP amplitude was used to calculate the area under the recruitment curve (AURC) for each time point. The curve was bounded by TMS intensity using the trapezoidal rule (Carson et al. 2013, Potteiger et al. 2002). More specifically, the following formula was used, $\sum \frac{20(a+b)}{2}$, where a and b represent MEP amplitudes at consecutive stimulus intensities, e.g., 90% rMT and 110% rMT. We also used short-latency intracortical inhibition (SICI) to assess $GABA_A$ receptor (Di Lazzaro et al. 2006) mediated intracortical inhibition and glutamatergic and intracortical facilitation (ICF) to examine N-methyl-D-aspartate (NMDA) receptor mediated intracortical facilitation (Ziemann et al. 1998). For the assessment of SICI and ICF, two Magstim units were configured to deliver paired pulse stimulation with an interstimulus interval (ISI) of 3 ms and 13 ms, respectively (Kossev et al. 2003, Kujirai et al. 1993). The intensity of the test TMS pulse (TS) was set to elicit an MEP of approximately 1.0 mV in the left FDI during rest, while the intensity of the conditioning stimulus (CS) was set at 80% of rMT. At each assessment time point (i.e., Pre 1, Pre 2, Post 0, Post 20, Post 40 and Post 24h), 12 TS and 12

CS-TS (3 ms ISI), and 12 CS-TS (13 ms ISI) were delivered in a random order while the participant remained at rest. SICI and ICF were expressed as ratios (conditioned MEP/ unconditioned MEP).

2.4 Transcranial direct current stimulation (tDCS)

Direct current was generated by a battery-driven constant-direct current stimulator (HDCStim class IIa; Model: HDCel EN-05, Newronika s.r.l., Milano 20122, Italy). The current was applied through two rubber electrodes that were placed inside pre-saline soaked and gelled sponges with conductive gel. For the stimulation during the task training, the positively charged electrode (anode) (5 cm x 5 cm) was placed over the left FDI hotspot (right M1) and the negatively charged electrode (cathode) (5 cm x 5 cm) was located over the contralateral supraorbital region. For the priming stimulation, the opposite montage was used, i.e., the cathodal electrode was placed over the left FDI hotspot and the anodal electrode over the contralateral supraorbital region. For sham stimulation, the stimulation set up had the same montage as the cathodal stimulation; However, the current was ramped down to zero over a period of 30 seconds (Nitsche et al. 2008). The cortical representation of the left (non-dominant) FDI was selected as the target region for tDCS because of the greater likelihood of observing motor improvements in the non-dominant hand. For priming tDCS with the cathode placed over M1, a constant current of 1.5 mA was delivered for 10 minutes, while for tDCS with the anode placed over M1 the same current intensity was applied for 20 minutes *during* the motor training (see more details in "ISOMETRIC FORCE CONTROL TASK (FORCE)" section). There is a growing body of literature to suggest that a current intensity of 1.5 mA applied during tDCS to M1 is effective at improving behavioral performance in tasks including reaction time (Karok and Witney 2013), force endurance (Cogiamanian et al. 2007), and sequence learning in healthy young adults. Indeed, Cuypers and colleagues (2013) recently reported that a current of 1.5 mA tDCS with the anode placed over M1 was more effective at improving retention in a sequence learning task than sham stimulation, while 1 mA tDCS with the anode placed over M1 failed to exhibit any gains above those observed with sham stimulation. At neurophysiological level, tDCS placing anode electrode placed over M1 with the current intensity of 1.5 mA increases MEP amplitude in young (Karok and

Witney 2013, Tremblay et al. 2013a, Williams et al. 2013) and older adults (Puri et al. 2016, Puri et al. 2015).

All participants and experimenters were blind regarding the nature (sham vs tDCS with the cathode placed over M1) of the tDCS priming. To this end, we highlight the specific features of tDCS which make it a preferred NIBS technique: Firstly, sham tDCS conditions can easily be applied such that both participant and experimenter are 'blind' (Gandiga et al. 2006). This particular facet permits completely unbiased assessment of measures (using double-blind sham-controlled protocols), as is required in strict trials of neurorehabilitation (Day and Altman 2000). Secondly, tDCS is significantly cheaper and smaller than apparatus used for rTMS and is very simple to use. The electrodes placement requires minimum experience. Accordingly, should the technique be found to be beneficial as a clinical tool, it would be relatively straightforward to transfer to clinical settings.

2.5 Isometric force control task (FORCE)

We used a sequential visual isometric index finger abduction task, henceforth referred to as the FORCE task (Saucedo Marquez et al. 2013). The FORCE task is a modified version of a task developed by Reis and colleagues (Reis et al. 2009). Participants sat in an armchair, positioned 60 cm from a 20 inch screen monitor, with their left forearm placed on a horizontal board at a table situated in front of them. The left palm faced down with the elbows slightly bent (100-120°). Vertical wooden pegs designed to restrict movements to the second metacarpophalangeal joint (Carroll et al. 2008, Lee et al. 2010), inserted into the board helped participants to maintain a consistent posture with hand and forearm muscle relaxed throughout the experiment (Hinder et al. 2012, Hinder et al. 2011).

Using their left index finger, participants were instructed to control a cursor (green square) displayed on the PC monitor by applying force on a force transducer (Load cell model 1042, TEDEA Huntleigh, USA) by isometrically abducting their left index finger. The green cursor represented the force level and corresponded to the width of 5 virtual units (VU). The task was to move the green

square between a home position (force 0) and nine target zones (flanked with vertical dotted lines) that were displayed in a fixed order (6-3-1-7-2-9-5-8-4).

The targets were equally distributed over the screen which corresponded to 100 virtual units (VU). The force was non-linearly transduced into the displacement of the green cursor using a formula; screen position = a x ln (force) + b, where a and b were adjusted for each participant in a way that reaching the furthest target required 40% of the maximal voluntary contraction (MVC). MVC was measured prior to the first FORCE test (Pre 1) taking the greatest value of three isometric left index finger abduction trials.

2.5.1 $FORCE_{test}$

At each assessment time point, participants performed 2 trials each at 6 different speeds (0.5, 0.75, 1, 1.33, 1.67, 1.83 Hz) in a random order. For example, at 1 Hz participants were instructed to move the cursor to a new gate in every second. During a trial, participants were instructed to isometrically abduct their left index finger against the force transducer to move the cursor as quickly and as accurately as possible back and forth between the home position and a target gate (i.e., Home-6-Home-3- Home-1- Home-7 Home-2- Home-9- Home-5- Home-8- Home-4). Participants were instructed to complete a cycle (i.e., move the cursor to the target and return to home) in time with an auditory metronome.

2.5.2 $FORCE_{training}$

The training time was divided into nine blocks, whereby the participants performed the FORCE task for 2 minutes followed by 1 minute of rest, having a total duration of 27 min. The execution speed was self-determined as a new target zone was displayed only after the green cursor came back to the home position after each attempt to locate the cursor within the specified target zone.

2.7 Questionnaires

Participants reported the number of hours of sleep and the quality of sleep in the night before the session on a 10 point scale. In addition, we asked for the number of units of alcohol and caffeine

intake in the last 12 hours before the session. Table 1 summarizes the means (M) and standard deviations (SD) for each item by group.

After the $FORCE_{training}$ with tDCS with the anode placed over M1, using a questionnaire developed by Brunoni et al. (2011) and Fertonani et al. (2010), we asked participants to report the presence and severity (none-mild-moderate-considerable-strong) of feelings of itchiness, tingling, headache, neck pain, scalp pain, burning, warmth/heat, pinching, iron taste, fatigue, concentration difficulties and acute mood changes. In addition, the start (beginning, middle or end of stimulation) and duration (stopped soon, in the middle or towards the end of the stimulation) of these sensations was questioned and participants were asked if the sensations influenced their performance (not at all, a little, considerably, much, very much). The mean tDCS sensation score is presented in Table 1.

2.8 Data processing and analysis

Following previous studies (Reis et al. 2009, Saucedo Marquez et al. 2013), a skill measure (skill index, SI) which reflects a shift in the task's speed-accuracy trade-off function was utilized in the current study. Such a parameter is very useful to quantify skill learning because it enables us to compare performance between trials which involve different speed and accuracy features, e.g., fast movements with many errors and slow movements with few errors. The consideration of speed-accuracy trade-off is particularly important in the context of aging since older adults achieve a similar level of motor performance with slower speed in comparison to young adults (e.g., Fujiyama et al. 2013, Heuninckx et al. 2004).

As in previous studies (Lopez-Alonso et al. 2015, Reis et al. 2009, Saucedo Marquez et al. 2013), we determined the speed-accuracy tradeoff of FORCE empirically. By pacing each subject at different movement frequencies, we can model the associated changes in accuracy. For each movement frequency, we obtained an error rate which is the proportion of trials with at least one over- (the force level exceeds the target gate) or under-shoot (the force level did not reach the target gate) movement, i.e., Skill index, which considers speed-accuracy trade-off by considering movement duration and accuracy, was modeled according to the formula:

$$Skill\ index = \frac{(1 - error\ rate)}{(error\ rate)(ln(duration)^b)}$$

, where *b* is the dimension free parameter, *error rate* is the average error within a trial, and *duration* is the movement time. Although Reis and colleagues (2009) used a constant b-value of 5.424 based on results obtained from a small control group, we opted to calculate b-values for each individual participant based on post-training data (i.e., Post 0, Post 20, Post 40, Post 24h) (Saucedo Marquez et al. 2013). Figure 2 illustrates the shifts in accuracy and speed in each stimulation group for young and older adults.

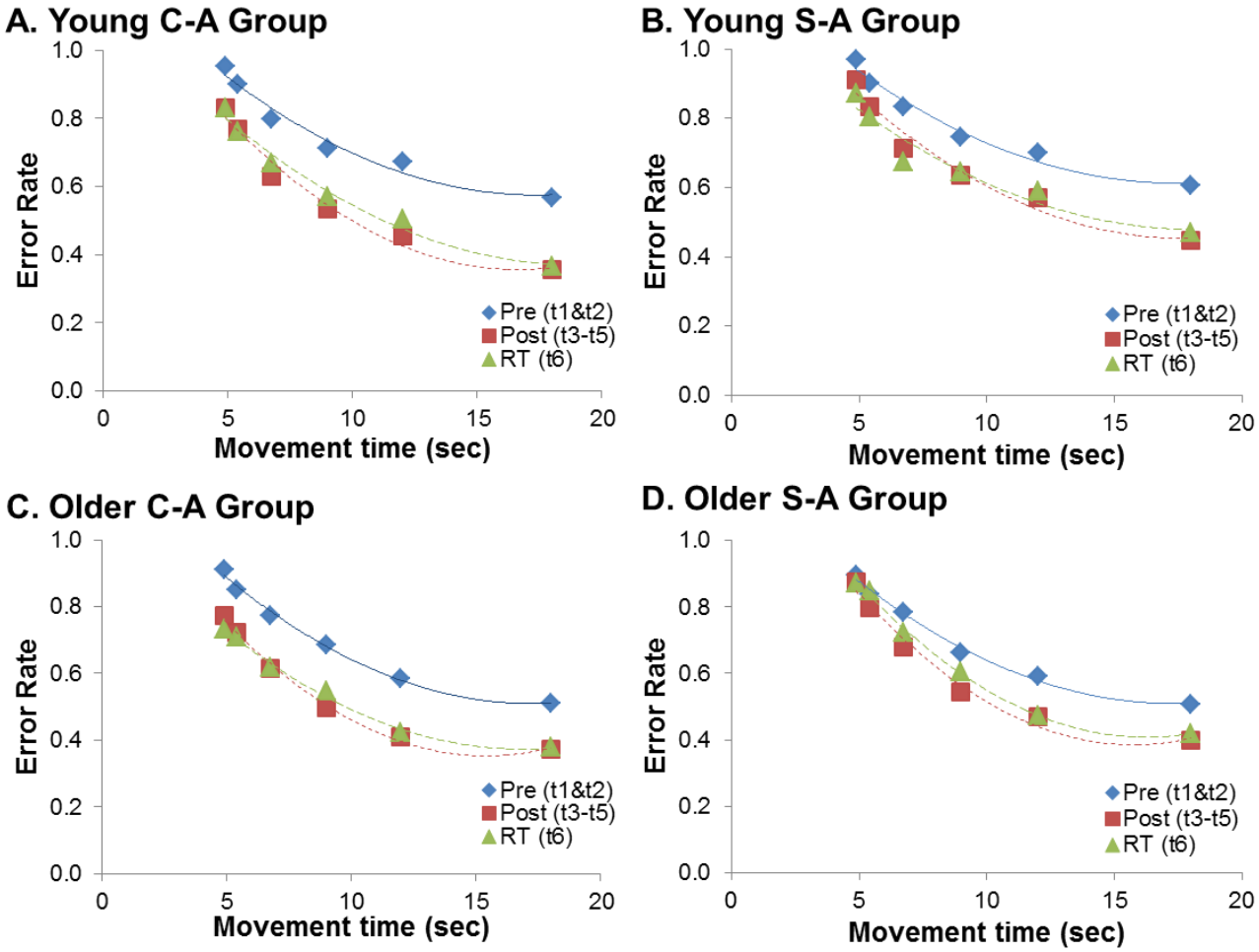

Figure 2. Speed-Accuracy Tradeoff Function for each AGE GROUP (young and older adults) and STIM GROUP (C-A and S-A). Blue diamond represents the pre data set (average across Pre1 and Pre2), red square represents post data (average across Post0, 20, and 40) and the green triangle represents the retention data (Post 24h).

2.9 Statistical analysis

Data are expressed as mean (M) ± 95% confidence intervals (CI) and were tested to ensure normality (Kolmorogov-Smirnov test) prior to parametric tests. Demographic measures were analyzed using 2 (AGE GROUP: young, older) x 2 (STIM GROUP: C-A, S-A) repeated measures ANOVAs. Resting motor thresholds (rMT) and 1mV TS intensity, both expressed as a percentage of maximum stimulator output (%MSO) were analyzed by 2 (AGE GROUP: young, older) x 2 (STIM GROUP: C-A, S-A) x 2 (SESSION: session 1 and session 2) repeated measures ANOVAs. Skill index obtained from $FORCE_{test}$ and TMS measures were normalized for each participant to the mean value obtained at Pre 1 to quantify the extent of changes from the Pre 1. As such, a normalized value larger than 1 reflects an increase in the measure relative to the Pre 1. Normalized skill index and TMS data (AURC, SICI, and ICF) were analyzed using 2 (AGE: young, older) x 2 (STIM: C-A, S-A) x 5 (TIME: Pre2, Post0, Post20, Post40, Post 24h) repeated measures ANOVAs. Additionally, the specific effect of tDCS with cathode placed over M1 was analyzed using a 2 (AGE GROUP: young, older) x 2 (STIM GROUP: C-A, S-A) x 2(TIME: Pre 1, Pre 2) ANOVA.

We also examined whether there were any relationships between neurophysiological changes and performance improvement. Using the normalized values, Pearson product-moment correlation coefficients were computed to assess the relationship between the performance changes and neurophysiological changes at each time point in the two stimulation conditions (S-A and C-A) for each age group separately.

Inter-individual variability in response to tDCS was also assessed. We performed chi-square tests to evaluate the percentage of responders and non-responders in two stimulation groups (i.e., S-A and C-A) divided on the basis of age or sex. Non-responders were defined operationally according to the ratio between pre (Pre2) and average of all post tDCS with the anode placed over M1 AURC values across time points (Post0, 20, and 40) being below 1.1, resulting to categorize those who showed a 10% increase in post tDCS with the anode placed over M1 during training as responders (Goldsworthy et al. 2016). We chose this criterion to accept more than 10% increases in AURC (ratio > 1) as physiologically meaningful (Hinder et al. 2014).

For ANOVA, if the sphericity assumption was violated ($\varepsilon < 0.7$) then we applied Huynh-Feldt degrees of freedom adjustment. For post-hoc analysis Tukey HSD was used, as necessary. The level of significance (p-value) was set at 0.05. Phi (φ) and partial eta-squared (η_p^2) values are provided as measures of effect size, where appropriate. Cut-offs ≥ 0.1 small, ≥ 0.3 medium, ≥ 0.5 large were applied for Phi φ, ≥ 0.01 small, ≥ 0.06 medium, and ≥ 0.14 large were applied for η_p^2 , and $\geq |0.10|$ small, $\geq |0.30|$ medium, and $\geq |0.50|$ large were applied for r (Sink and Stroh 2006).

3. Results

3.1 Force performance

Skill index. To meet the assumption of normality a log transformation was applied to the skill index data. For clarity, non-transformed data are reported in the text and figures. ANOVA revealed main effects of AGE, $F(1, 56) = 17.08$, $p < 0.001$, $\eta_p^2 = 0.23$, STIM, $F(1, 56) = 6.59$, $p = 0.013$, $\eta_p^2 = 0.11$, and TIME, $F(4, 224) = 56.45$, $p < 0.001$, $\eta_p^2 = 0.50$. These main effects were best interpreted with reference to the significant interaction between AGE and TIME, $F(4, 224) = 3.50$, $p = 0.008$, $\eta_p^2 = 0.06$, and a significant interaction between STIM and TIME, $F(4, 224) = 5.61$, $p < 0.001$, $\eta_p^2 = 0.09$. Post-hoc comparisons for the interaction of AGE and TIME showed that the skill index at all post time points was significantly higher than at Pre2 in both young and older adults (all $p < 0.001$). Additionally, in young adults the skill index at Post40 was significantly higher than at Post0 ($p = 0.001$). The interaction was driven by the lower skill index values in older adults following tDCS with the anode placed over M1 during $FORCE_{training}$ (all $p < 0.05$) relative to young adults (at all post-training time points), suggesting that older adults showed reduced skill learning ability compared to young adults.

Post-hoc comparison for the interaction between STIM and TIME revealed that both STIM groups showed skill improvement following tDCS with the anode placed over M1 during $FORCE_{training}$ (all $p < 0.05$). C-A group showed significantly higher skill index scores at Post0 ($p = 0.01$) and Post40 ($p = 0.04$) relative to the S-A group (Figure 3). One other critical aspect of these results was that there were no differences in skill changes between C-A and S-A group following

priming tDCS with the cathode placed over M1, suggesting that 10 min of tDCS with the cathode placed over M1 did not have an apparent effect on skill acquisition, but indeed primed the system to subsequent training and for tDCS with the anode placed over M1 to magnify the gain. Of note, the three-way interaction between AGE, STIM, and TIME was not significant, $F(4, 224) = 1.00$, $p = 0.39$, $\eta_p^2 = 0.09$.

In sum, these results indicate that older adults demonstrated a reduced ability to acquire a new skill relative to young adults. However, for both age groups, the application of priming tDCS with the cathode placed over M1 induced greater learning within subsequent $FORCE_{training}$ conducted with simultaneous tDCS with the anode placed over M1 compared to the motor learning exhibited when priming tDCS was not applied.

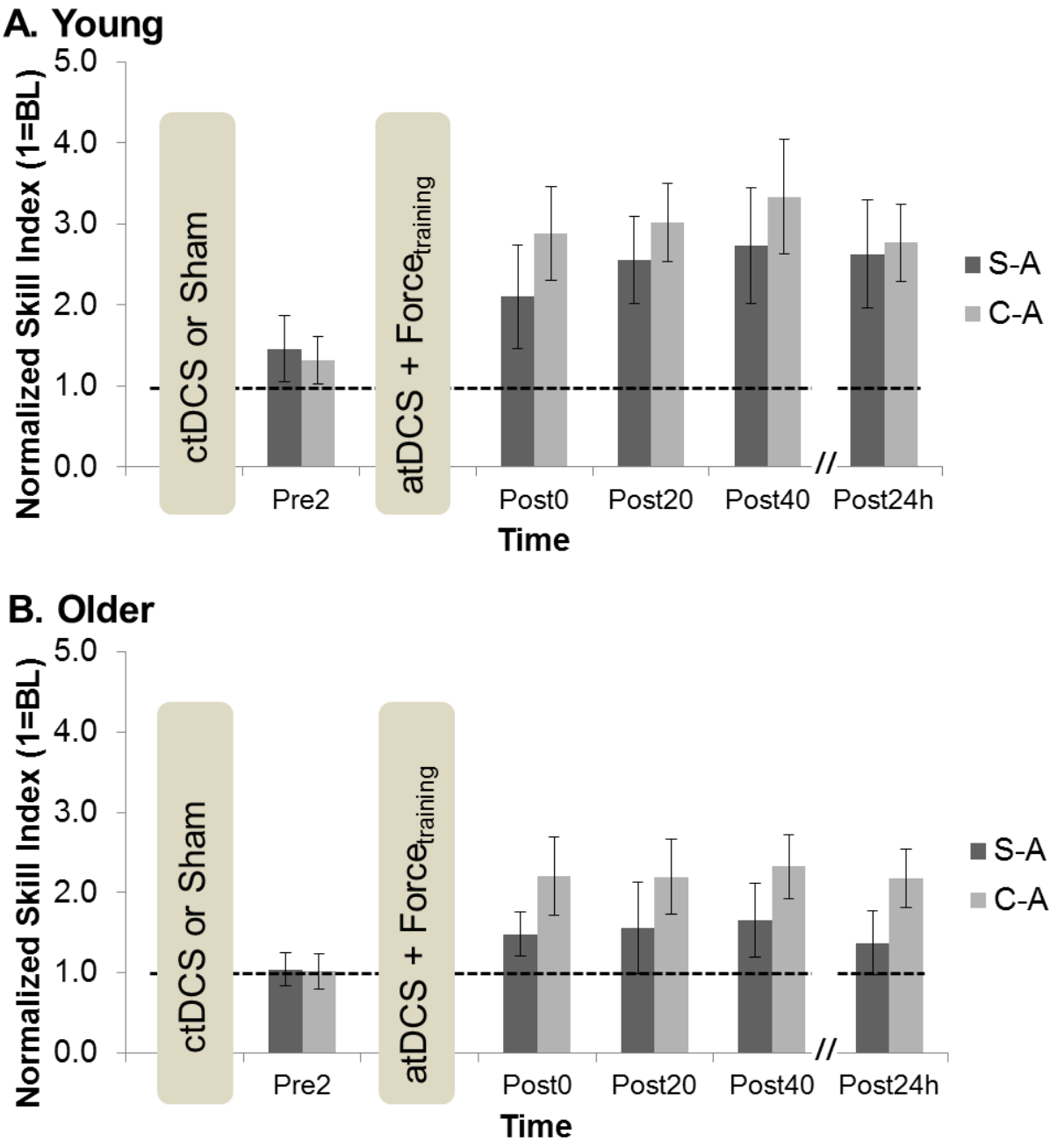

Figure 3. Mean normalized skill index for A) Young and B) Older adults at each time point. All error bars (95% CI) which include the value 1 (baseline: dotted horizontal line) indicate non-significant differences at that time relative to the baseline condition. Asterisks denote significant differences between groups ($p < 0.05$).

3.2 TMS measures

Resting motor threshold (rMT). ANOVA revealed no significant main effects or interaction, Fs < 2.75, ps > 0.10, η_p^2s < 0.05, suggesting that rMT did not vary as a function of session (Session1: M =

40.98 ± 2.68; Session 2: *M* = 41.42 ± 2.67), age (young: *M* = 39.72 ± 2.32; older: *M* = 42.68 ± 2.89), or stimulation condition (S-A group: *M* = 41.15 ± 3.01; C-A group: *M* = 41.25 ± 2.30).

1mV TS. Although higher TS intensity was required to elicit 1 mV MEP amplitude in older adults (*M* = 65.34 ± 3.84; corresponding to approx. 135% rMT) relative to young adults (*M* = 52.01 ± 2.90; corresponding to approx. 116% rMT), the main effect of AGE just failed to reach conventional significance level, $F(1, 56) = 3.86$, $p = 0.054$, $\eta_p^2 = 0.06$. There were no other main effect or interactions, all $F < 1.50$, all $p > 0.23$, all $\eta_p^2 < 0.03$, suggesting that 1 mV TS intensity did not vary significantly as a function of session (Session1: *M* = 58.48 ± 4.09; Session 2: *M* = 58.90 ± 3.98), age, or stimulation group (S-A group: *M* = 58.33 ± 4.43; C-A group: *M* = 59.05 ± 4.02).

Corticospinal excitability (AURC). To meet the assumption of normality a log transformation was applied to the AURC data. For clarity, non-transformed data are reported in the text and figures. The effect of tDCS with cathode placed over M1 was examined using a 2 (AGE GROUP: young, older) x 2 (STIM GROUP: C-A, S-A) x 2(TIME: Pre 1, Pre 2) ANOVA. There were no significant main effects or interactions, all $F < 1.84$, all $p > 0.18$, all $\eta_p^2 < 0.03$, indicating that corticospinal excitability did not change after tDCS with cathode placed over M1 in both young and older adults.

To investigate the overall changes in AURC, normalized AURC data were analyzed using 2 (AGE: young, older) x 2 (STIM: C-A, S-A) x 5 (TIME: Pre2, Post0, Post20, Post40, Post 24h) repeated measures ANOVAs. There were significant effects of STIM, $F(1, 56) = 4.05$, $p = 0.049$, $\eta_p^2 = 0.07$, and TIME, $F(4, 224) = 8.64$, $p < 0.001$, $\eta_p^2 = 0.13$, which are best interpreted with reference to the significant interaction between STIM and TIME, $F(4, 224) = 2.79$, $p = 0.03$, $\eta_p^2 = 0.05$. As shown in Figure 4A and B, in the C-A group (for both young and older adults), AURC values were significantly higher at all time points following tDCS with the anode placed over M1 and $FORCE_{training}$ (Post0, Post20, Post40) relative to Pre2 (*p*s < 0.05). Furthermore AURC at Post20 and 40 was significantly higher than Post24h (*p*s < 0.01) across both age groups. In contrast, the S-A groups (for both young and older adults) did not show significant changes in AURC values across time points. Furthermore, AURC values at Post20 and Post40 in the C-A group were significantly

higher than S-A group (all $p < 0.03$). These results suggest that corticospinal excitability was significantly potentiated following tDCS with the anode placed over M1 with $FORCE_{training}$ in the group receiving priming tDCS with the cathode placed over M1 (i.e., C-A group), but not in the group receiving anodal stimulation over M1 with $FORCE_{training}$ (i.e., S-A group) without priming tDCS with the anode placed over M1. Importantly, there was no main effect of AGE or interactions including AGE as a factor, all $F < 2.72$, all $p > 0.10$, all $\eta_p^2 < 0.05$, suggesting that the modulation of corticospinal excitability following stimulation with $FORCE_{training}$ did not vary significantly as a function of age. With respect to the effect of priming tDCS with the cathode placed over M1, similar to skill acquisition, 10 min of tDCS with the cathode placed over M1 at 1.5 mA did not overtly change corticospinal excitability in both young and older adults (95% CI error bars at Pre2 include baseline (Pre1)(Figure 4).

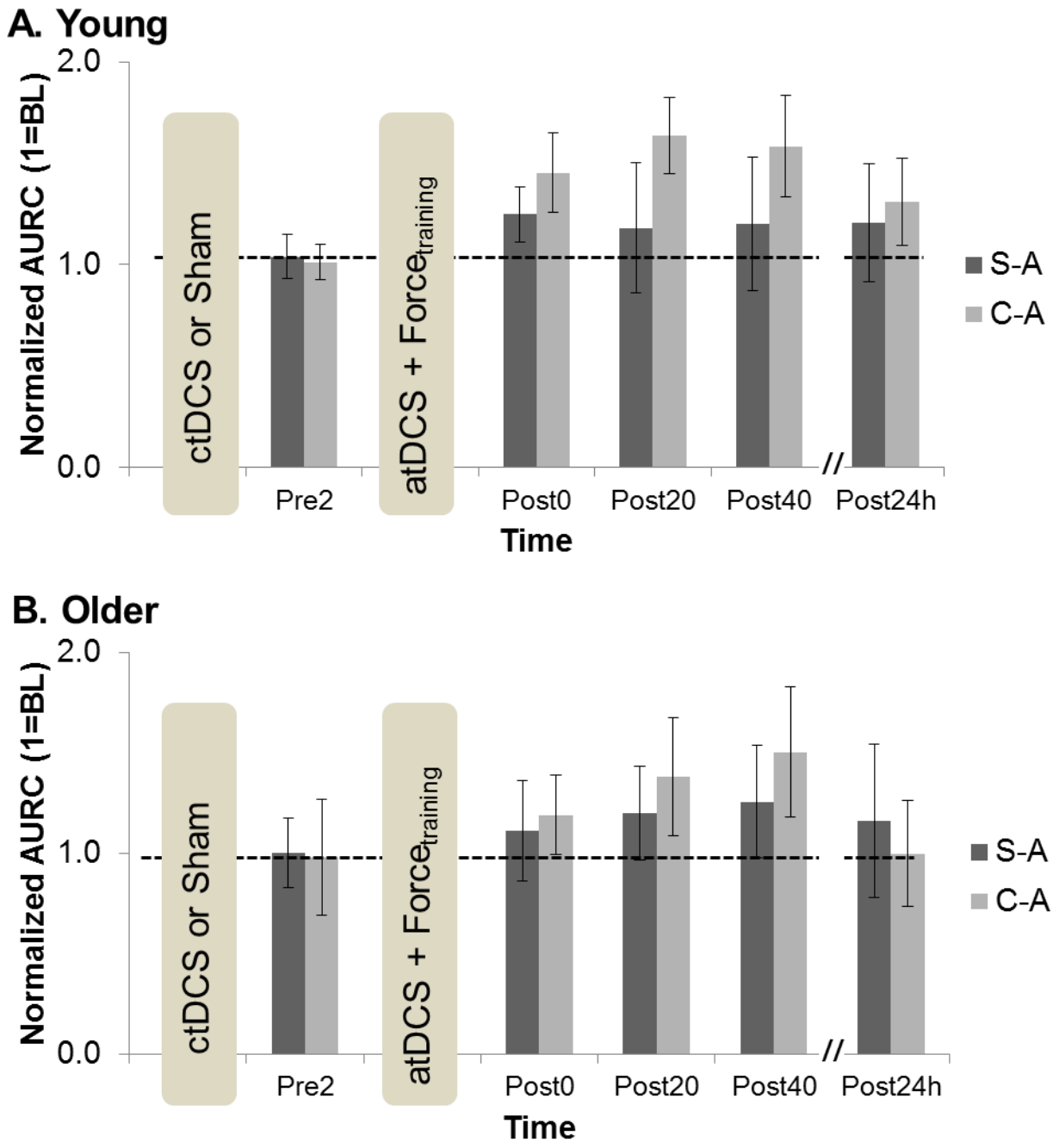

Figure 4. Mean normalized AURC values for A) Young and B) Older adults at each stimulation time point. All error bars (95% CI) which include the value 1 (baseline: dotted horizontal line) indicate non-significant differences at that time relative to the baseline resting condition. Asterisks denote significant differences between groups ($p < 0.05$).

Short-latency intracortical inhibition (SICI). Non-normalized SICI values indicate that young (0.57 ± 0.05) and older adults (0.67 ± 0.16) exhibited levels of inhibition at Pre1 that did not vary significantly (independent *t*-test, $p = 0.17$). ANOVA to assess changes in SICI induced by tDCS with the anode

placed over M1 during training (i.e., SICI normalized to Pre 1 revealed no significant main effects or interactions, Fs < 1.56, ps > 0.19, η_p^2s < 0.03.

Intracortical facilitation (ICF). An independent *t*-test on raw (non-normalized) ICF values revealed that young adults (1.86 ± 0.16) had greater ICF compared to older adults (1.46 ± 0.15) at Pre1 (p = 0.005). As with normalized SICI, ANOVA to assess changes in ICF (ICF normalized to Pre 1) revealed no significant main effects or interactions, Fs < 1.85, ps > 0.18, η_p^2s < 0.03.

3.3 Association between skill acquisition and neurophysiological changes

In young adults, there were no significant correlations between skill changes and changes in TMS measures at any time point. In contrast, older adults in both stimulation groups showed significant positive correlations between skill changes and AURC changes at Post0, Post40, and Post24h (Table 1, Figure 5). Although correlation coefficients at Post20 in older adults of both stimulation groups did not reach significance, these correlation coefficients achieved medium effect sizes (rs > 0.3). No significant correlations were evident for SICI and ICF. In sum, these results suggest that in older adults, greater changes in corticospinal excitability following tDCS with the anode placed over M1 during training were associated with more pronounced skill acquisition.

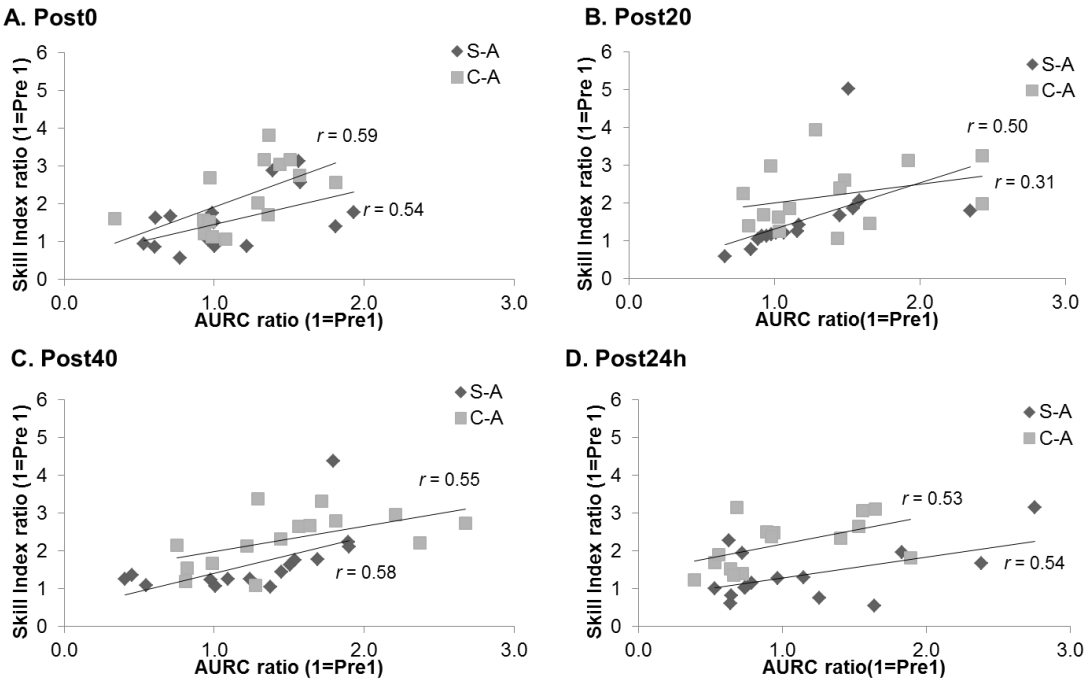

Figure 5. Association between changes in corticospinal excitability (indexed by AURC change relative to Pre1) and degree of skill acquisition (indexed by skill index change relative to Pre1) at A) Post0, B) Post20, C) Post40, and D) Post24h in older adults. *Note*: Bold *r* values depict significant correlation coefficients (critical $r = \pm .51$).

3.4 Inter-individual variability of responses to tDCS

We further investigated whether the application of tDCS with the cathode placed over M1 prior to tDCS with the anode placed over M1 reduces the inter-individual variability in neurophysiological measures. Since we only observed modulations in AURC, but not in other TMS measures (SICI and ICF) following tDCS with the anode placed over M1 during training, we only consider AURC for this analysis. In line with previous work (e.g., Fujiyama et al. 2014), some individuals did not display the anticipated increase in AURC following tDCS with the anode placed over M1 during FORCE training (Figure 6). In the S-A group, 40 % of total participants, specifically 6 young (4 females) and 6 older (3 females) adults, were identified as non-responders exhibiting average AURC values across all post tDCS with the anode placed over M1 time points of less than 1.1 (i.e., less than 10% increase in AURC). In contrast, all young, except one female and 11 older participants (5 males and 6 females) in the C-A group exhibited AURC values larger than 1.1 following tDCS with the anode placed over M1 during training. Response to tDCS with the anode placed over M1 did not differ by age, $\chi^2(1, N = 60)$

= 1.00, $p = 0.51$, $\varphi < 0.13$, or sex, $\chi^2(1, N = 60) = 0.208$, $p = 0.65$, $\varphi = 0.06$. Importantly, however, a significantly larger number of participants were identified as responders to tDCS with the anode placed over M1 in the C-A group compared to the S-A group, $\chi^2(1, N = 60) = 4.02$, $p = 0.04$, $\varphi = 0.26$.

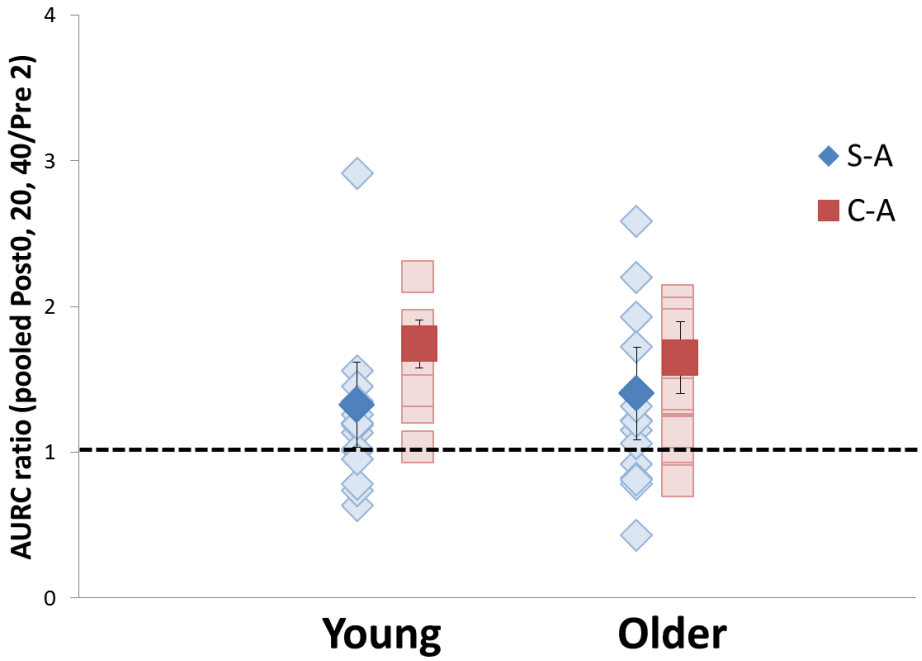

Figure 6. Mean (filled square C-A group and filled diamond for S-A group, error bars denotes 95% CI and individual AURC ratios (pooled AURC across Post0, 20, and 40 divided by baseline Pre2) in young and older adults. A ratio larger than 1 indicates AURC increases following tDCS with the anode placed over M1 during FORCE training.

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4. Discussion

The present study was designed to investigate the effect of tDCS with the anode placed over M1 during motor learning when it was preconditioned (i.e., primed) by tDCS with the cathode placed over M1. Although the extent of skill acquisition during the application of tDCS with the anode placed over M1 was reduced in older adults compared to young adults (e.g., Pauwels et al. 2015, Zimerman et al. 2013), priming this tDCS with the anode placed over M1 during motor learning with tDCS applying the cathode to M1 resulted in greater skill improvement than priming with sham tDCS for both young and older adults. Similarly, for both age groups, corticospinal excitability changes induced by the tDCS with the anode placed over M1 during skill training protocol were also greater and more

reliable (across participants) following real compared to sham priming tDCS with the cathode placed over M1. Notably, correlation analyses suggested that greater increases in corticospinal excitability were associated with more training-induced improvement, but only for older adults.

4.1 Behavioral effects of tDCS with the cathode placed over M1 prior to combined tDCS with the anode placed over M1 and motor learning

Although skill gain was reduced in older adults compared to young adults, the level of skill gain was commensurate in young and older adults, suggesting that older adults did not show age-related reductions in the ability to learn a fine motor skill when it was undertaken concurrently with tDCS placing the anode over M1. This result confirms previous work reporting that older adults maintain the ability to learn new motor skills (Seidler 2007, Swinnen et al. 1998). Previous studies have demonstrated the effect of tDCS with the anode placed over M1 on motor behaviour when the stimulation was applied during training of a functional motor task (Hummel et al. 2010), sequence learning (Zimerman et al. 2013), and visuo-motor tracking tasks (Goodwill et al. 2015, Goodwill et al. 2013). Here we demonstrated that tDCS with the anode placed over M1 also improved a unimanual isometric force control task (Reis et al. 2009, Saucedo Marquez et al. 2013) in both age groups. Importantly, the skill acquisition in both older and younger adults was significantly improved when priming tDCS with the cathode placed over M1 was administered prior to the tDCS with the anode placed over M1 during motor training. Recently, the application of tDCS with the cathode placed over M1 prior to tDCS with the anode placed over M1 in healthy young adults resulted in a greater improvement in a functional motor task performance (grooved pegboard test) relative to tDCS with the anode placed over M1 preceded by sham stimulation (Christova et al. 2015). Accordingly the current study, for the first time, reveals that tDCS with the anode placed over M1 during skill training in combination with preceding tDCS with the cathode placed over M1 is also effective in facilitating motor learning in older adults.

4.2 Neurophysiological effects of tDCS with the cathode placed over M1 prior to combined tDCS with the anode placed over M1 and motor learning

The current study demonstrated that plastic changes in the corticospinal system largely follow the rule of homeostatic metaplasticity by showing greater corticospinal excitability increases in the C-A group compared to S-A group, which is in agreement with previous studies using tDCS (Christova et al. 2015) and other forms of NIBS protocols in healthy young adults (Iyer et al. 2003, Muller et al. 2007, Murakami et al. 2012, Siebner et al. 2004).

In view of the fact that tDCS may be an important adjunct to motor training rehabilitation programs, an important novel finding of the current study is that older adults showed comparable corticospinal excitability increases to those exhibited by young adults. This suggests that, at least at the neural level, the ability to undergo plastic changes in the central nervous system is maintained in healthy aging. Another important aspect of the current results is that the extent of potentiation in corticospinal excitability was positively correlated with skill changes in both groups of older adults (i.e., C-A and S-A), suggesting that the responsiveness of the corticospinal system to external stimuli (combined tDCS and motor training) is possibly instrumental in driving short-term skill acquisition in older adults.

Interestingly, the application of tDCS with the cathode placed over M1 (10 min, 1.5mA) did not induce overt decreases in corticospinal excitability or performance decline in the FORCE task. In fact, the lack of changes in corticospinal excitability following tDCS with the cathode placed over M1 over M1 is in agreement with recent studies (Strube et al. 2016, Wiethoff et al. 2014). In this respect, the tDCS with the cathode placed over M1 protocol in the current study acted to amplify the effect of subsequent tDCS with the anode placed over M1 during skill training on performance improvement without overtly increasing corticospinal excitability, suggesting the priming nature of the particular protocol. Priming with a relatively short application of tDCS (10 min, 1.5mA) with the cathode placed over M1 is thought to lower neuronal activity to reduce the threshold for subsequent tDCS with the anode placed over M1 to increase corticospinal excitability (Siebner et al. 2004, Ziemann and Siebner

2008). This priming or pre-conditioning effect likely resulted in the greater increases in corticospinal excitability (and improved skill acquisition during training) in the C-A group relative to the S-A group. Furthermore, the significantly lower number of non-responders in the C-A groups relative to the S-A groups not only further supports this view, but also provides a good basis for the application of tDCS as a promising intervention tool to assist acquiring novel skills in older adults by reducing inter-individual variability in response to tDCS.

The priming effect observed here with tDCS with the cathode placed over M1 is consistent with the priming effect obtained following application of a continuous theta burst stimulation protocol (cTBS), a form of repetitive TMS (rTMS). The standard cTBS protocol involves delivery of trains of three subthreshold stimuli at 50 Hz every 200 ms for 20 or 40 sec (for a total of 300 or 600 pulses – cTBS300 or cTBS600, respectively), which elicit decreases in corticospinal excitability when applied over M1 (Huang et al. 2005). Notably, a shorter duration of cTBS involving 150 pulses (cTBS150) – without itself inducing overt changes in corticospinal excitability – reverses the subsequent effect of cTBS300 from the expected LTP-like effects to LTD-like effects of cTBS300 (Huang et al. 2010). Using a cTBS150 priming protocol, Canterero and colleagues (2013) elucidated the interaction of motor learning and occlusion of LTP-like effects on MEP amplitude following skill training. Specifically, the expected corticospinal excitability increase following skill learning was abolished by the application cTBS150 (see Hinder et al. 2013 for a short review). Accordingly, the results of the study by Cantarero and colleagues (2013) together with the results of the tDCS with the cathode placed over M1 protocol in the current study provides strong evidence that short applications of NIBS that do not overtly alter corticospinal excitability, have the capacity to prime (e.g., significantly modulate or even reverse) the expected effect of subsequent LTP/LTD-like inducing protocols such as motor learning or NIBS.

The current study also examined SICI and ICF to elucidate changes in $GABA_A$ (Di Lazzaro et al. 2006) and glutamatergic N-methyl-D-aspartate (NMDA) receptors (Ziemann et al. 1998) following tDCS/motor learning, respectively. A magnetic resonance spectroscopy study by Stagg and colleagues

(2011a) revealed that tDCS (1mA, 10min) with the anode placed over M1 reduces local GABA concentration and tDCS with the cathode placed over M1reduces glutamatergic activity. In a subsequent study, Stagg and colleagues (2011b) found significant correlations between GABA concentration level and 1 ms ISI SICI which is thought to reflect extrasynaptic GABA tone (Stagg et al. 2011b) as well as corticospinal excitability and glutamate level. However, there was no association between GABA concentration level and SICI with 2.5 ms ISI which is thought to reflect synaptic $GABA_A$ activity. Similarly, in the current study, neither SICI nor ICF showed any modulations in the course of the assessment. Although a number of studies have reported reduced SICI (i.e., released inhibition) following tDCS with the anode placed over M1 (Amadi et al. 2015, Cengiz et al. 2013, Christova et al. 2015, Heise et al. 2014, Kidgell et al. 2013), other studies report no change in SICI and ICF following tDCS with the anode placed over M1 (Siebner et al. 2004) or increased SICI and reduced ICF (Batsikadze et al. 2013). These apparent discrepancies may originate from the fact that both tDCS (duration, current intensity, electrode size) and TMS parameters (inter-stimulus interval, intensities of conditioning pulse) vary somewhat across the studies. Alternatively, SICI and ICF may not be the optimum TMS protocols to reflect changes in GABA and glutamatergic activity following tDCS, as Tremblay and colleagues (2013b) showed that only TMS-induced silent period duration was significantly correlated with glutamate and glutamine concentrations. Therefore, a comprehensive study controlling these parameters and considering a wide range of TMS measures is warranted in the future.

There are several potential limitations in the present study. Firstly, a group who received priming tDCS with the cathode placed over M1 followed by sham stimulation during training was not included in the current study. Such a group would have enabled us to directly investigate, for the $FORCE_{training}$ task, whether the priming protocol alone could elicit a level of skill acquisition during training that was comparable to the combination of priming together with tDCS with the anode placed over M1 during training. However, given that (a) a number of studies have consistently shown that skill training conducted with concurrent tDCS with the anode placed over M1 results in better learning

compared to skill training without tDCS with the anode placed over M1 (e.g., Christova et al. 2015) and (b) the tDCS with the cathode placed over M1 used as our priming protocol has been shown to have no observable effect on subsequent motor learning (Stagg et al. 2011c), it is highly likely that the greater skill gains and larger corticospinal excitability changes in the C-A group (compared to the S-A group) in the current study were facilitated by the application of tDCS with the anode placed over M1 during skill training. Second, at post 24h, there were no statistically significant group differences in skill index. While in older adults the primed group (C-A) had better skill scores compared to the non-primed group (S-A) at Post 24h (Figure 3B), in young adults the advantages gained by the primed group (C-A) in skill acquisition was not apparent at post 24h. In this respect, future studies involving stimulating participants over multiple days as in Reis et al. (2009), are warranted to establish the effectiveness of the priming approach for long-term retention of motor skill.

4.3 Conclusion

The application of priming tDCS with the cathode placed over M1 prior to the application of tDCS with the anode placed over M1 during motor training led to enhanced skill acquisition in younger and older adults. Particularly in older adults, improved skill acquisition during training was most likely driven by the increased corticospinal excitability following tDCS with the anode placed over M1 during training, which was more robust (i.e., greater change) and reliable (between participants) following the priming. Thus, it appears that priming optimises the corticospinal system for upcoming combined input of tDCS and motor training. This is the first empirical evidence that homeostatic metaplasticity may be utilised to promote new skill acquisition in older adults. Priming or preconditioning the corticospinal system using tDCS with the cathode placed over M1 prior to tDCS with the anode placed over M1 during training may be a promising intervention protocol to improve the impact of tDCS by reducing variability across subjects. Such protocols may serve to facilitate the functional independence of the aging population and could also be applied to intervention protocols for clinical populations such as stroke patients.

Disclosure statement

The authors have no conflicts of interest to disclose.

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Table captions

Table 1. Summary of demographic information and questionnaires

Table 1
Summary of demographic information and questionnaires

	Young		Older		
	C-A (n = 15)	S-A (n = 15)	C-A (n = 15)	S-A (n = 15)	p-values
Demographic information	*M*(*SD*)	*M*(*SD*)	*M*(*SD*)	*M*(*SD*)	
Age	25.3 (2.7)	25.5 (3.3)	68.0 (3.2)	68.0 (5.7)	-
Sex	6 M / 9 F	8 M / 7 F	7 M / 8 F	8 M / 7 F	-
sleep, alcohol and caffeine intake					
Sleep quality	7.7 (1.8)	7.4 (1.1)	7.1 (2.5)	7.6 (2.3)	> 0.46
Sleep duration	6.7 (1.8)	7.2 (1.1)	6.6 (1.6)	6.7 (1.7)	> 0.52
alcohol intake	0.6 (0.9)	0.6 (0.7)	0.5 (1.6)	0.6 (1.4)	> 0.43
caffeine intake	0.2 (0.4)	0.0 (0.0)	0.3 (0.6)	0.5 (1.1)	> 0.16
tDCS questionnaire					
tDCS sensation	0.2 (0.4)	0.3 (0.4)	0.2 (0.1)	0.2 (0.2)	> 0.15