RUNNING HEAD: Enhancement of motor memories

Title: Brain polarization enhances the formation and retention of motor memories

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Abstract
One of the first steps in the acquisition of a new motor skill is the formation of motor memories. Here we tested the capacity of transcranial direct current stimulation (tDCS) applied over the motor cortex during motor practice to increase motor memory formation and retention. Nine healthy individuals underwent a crossover transcranial magnetic stimulation (TMS) study designed to test motor memory formation resulting from training. Anodal tDCS elicited an increase in the magnitude and duration of motor memories in a polarity-specific manner, as reflected by changes in the kinematic characteristics of TMS-evoked movements after anodal, but not cathodal or sham stimulation. This effect was only present when training and stimulation were associated, and mediated by a differential modulation of corticomotor excitability of the involved muscles. These results indicate that anodal brain polarization can enhance the initial formation and retention of a new motor memory resulting from training. This process may be the underlying mechanisms by which tDCS enhances motor learning.
Introduction

The ability to learn an unlimited repertoire of motor skills is one of the main characteristics of human beings. Recent studies have suggested that the primary motor cortex (M1) is crucially involved in this ability by forming and retaining short-term memory representations of recently practiced movements (Classen et al. 1998; Hadipour-Niktarash et al. 2007; Molina-Luna et al. 2008; Muellbacher et al. 2002). In particular, it has been shown that while disruption of M1 activity can interfere with retention of practiced motor tasks (Hadipour-Niktarash et al. 2007; Muellbacher et al. 2002) enhancing M1 excitability with transcranial direct current stimulation (tDCS) can improve it (Reis et al. 2009). However, it is not clear whether this beneficial effect on retention is mediated by larger amount of motor memory formation or changes in retention mechanisms.

One of the initial steps in the acquisition of new complex motor skills is the formation of motor memories (Classen et al. 1998; Muellbacher et al. 2002). Classen et al. (1998) used transcranial magnetic stimulation (TMS) over M1 to ascertain how simple motor memories are formed. In this paradigm, the preferred direction of TMS-evoked thumb movements is measured before and after performance of motor training. In this manner, it is possible to observe that training results in changes in the direction of TMS-evoked movements, indicative of formation of new motor memories containing the kinematic details of the practiced motions that is retrieved by the magnetic stimulation. This phenomenon is mediated by N-methyl-D-aspartate (NMDA), muscarinic, adrenergic and GABAergic neurotransmission (Butefisch et al. 2000; Sawaki et al. 2002b, 2003), and can be enhanced by dopaminergic (Floel et al. 2005a,b) and adrenergic agents (Butefisch et al. 2002, Sawaki et al. 2002a), D-amphetamine, congruent action observation (Stefan et al. 2008; Celnik et al. 2006) and hebbian-type non-invasive stimulation (Butefisch et al. 2004).

Transcranial direct current stimulation (tDCS) is a non-invasive, painless cortical stimulation technique (Nitsche and Paulus, 2000, 2001; Nitsche et al. 2003a,b, 2005).
While anodal tDCS results in increased cortical excitability without direct neuronal depolarization, cathodal tDCS decreases excitability (Purpura and McMurtry, 1965; Nitsche and Paulus, 2000; Nitsche et al. 2003). Interestingly, anodal tDCS appears to have a facilitatory effect on different forms of learning in healthy individuals (Antal et al. 2004; Floel et al. 2008; Kincses et al. 2004; Nitsche et al. 2003c; Reis et al. 2009), stroke (Hummel et al. 2005) and Parkinson patients (Boggio et al. 2006; For a review: Wu et al. 2007). However, it is not clearly understood what specific process mediates the enhanced learning when tDCS is applied during training. For instance, it is possible that the performance improvement described after learning a motor task under the influence of tDCS is mediated by increased memory formation, stronger retention of the formed memories, or better recall. Antal et al. (2004) and Nitsche et al. (2003c) have previously shown performance improvements during early learning, which suggests that tDCS may influence memory formation. In this study, we used a well-established paradigm that assesses the formation and retention of a motor memory without engaging voluntary recall to test the hypothesis that anodal tDCS over M1 enhances these processes relative to sham stimulation. In addition, in 3 separate controls we determine (1) the polarity specificity of the effects by testing cathodal stimulation, (2) the longevity of the effects by reassessing periodically the effects of stimulation and training, and (3) the association between motor practice and stimulation by testing the effects of anodal tDCS without training.

Method

Subjects

Nine right-handed (self-assessed) healthy individuals with no history of neurological or psychiatric conditions (mean ±SD 30 ±9 years old, 6 females) participated in the study. All subjects denied the use of acute or chronic CNS-acting medication, drinking alcohol in the previous 24 hours and reported to have had at least 5 hours of sleep the previous night. All subjects signed informed consent approved by Johns Hopkins Medical Institution Institutional Review Board and in accordance to the declaration of Helsinki.
Experimental Design

All subjects participated in two randomly ordered sessions separated by at least 5 days and designed to evaluate the effect of anodal tDCS on the encoding of motor memories (figure 1) (Classen et al. 1998).

Recordings and stimulation procedures
Subjects sat comfortably in a chair while the right forearm was restrained in a semi-pronated position with a molded cast with the four fingers in a slightly extended position and the thumb left entirely free. Using a two dimensional accelerometer (Kistler Instrument Corp, Amherst, NY) placed on the proximal phalanx of the thumb, movement directions were determined and calculated from the first peak acceleration vector in the vertical (extension/flexion) and horizontal axis (adduction/adduction) (figure 1) (Classen et al. 1998). Electromyographic (EMG) activity was recorded through pairs of disposable electrodes placed over the right extensor pollicis brevis (EPB) and right flexor pollicis brevis muscles (FPB). EMG signals were recorded, amplified and filtered (bandwidth 5 Hz-1KHz) with a Viking IVP (Nicolet, Viasys healthcare). All signals were sampled at 2 KHz, visually displayed online and stored for off-line analysis using a custom LabView program (National Instruments, Austin, Texas).

Transcranial magnetic stimulation (TMS)
Transcranial magnetic stimulation was delivered using a 70mm loop diameter figure-of-eight coil (Magstim® BiStim², Whitland, UK) placed over the left M1 to optimally activate the corticospinal tract and elicit focal isolated and directionally consistent thumb movements. Using a frameless neuronavigation system (BrainSight, Rogue Research, Montreal, Quebec, Canada) we co-registered the subjects’ heads to a standard MRI and marked this “Hot Spot”. We decided to use Brainsight as this is an accurate way to ensure the TMS coil remains in the same position within the X,Y,Z direction during and between each session. Resting motor threshold for the muscle agonist to the training (see below) was determined at this position and defined as the minimum TMS intensity that evoked
motor-evoked potentials (MEP) of 50 uV in at least five of ten trials (Rossini et al. 1994). Muscle relaxation was monitored by visual feedback of the EMG recording.

**Transcranial direct current stimulation (tDCS)**

Transcranial direct current stimulation (tDCS) was delivered through two sponge electrodes (surface area: 25cm²) embedded in a saline-soaked solution. Depending on the session, the anode or cathode was positioned on the marked motor “hot spot” and the other electrode on the skin overlying the contralateral supraorbital region. During training, 1mA anodal tDCS was delivered for approximately 30 minutes in the corresponding session, and up to 30 seconds in the sham session using a Phoresor II Auto (Model No.PM850; IOMED). At the onset and offset of tDCS, the current was increased or decreased in a ramp-like fashion, a method shown to achieve good blinding level (Gandiga, 2006).

**Experimental Procedure**

The formation of a motor memory was assessed using a previously described protocol (Butefisch et al. 2000; Celnik et al. 2006; Classen et al. 1998; Stefan et al. 2008). At the beginning of each session, 65 TMS stimuli were delivered at 0.1Hz over the hot spot to determine the baseline TMS-evoked thumb movement direction (Pre; figure 1). Subsequently subjects performed motor training consisting of voluntary thumb brisk movements paced by an audio metronome at 1Hz for 30 minutes in a direction opposite to the baseline TMS-evoked direction. For example, if the principal baseline direction was extension and abduction, then subjects were instructed to perform repetitive flexion and adduction movements (Figure 1 and 2). The participants were instructed to relax and let the thumb return to its original position after each motion. This was ensured by monitoring online EMG and acceleration signals and providing verbal feedback when needed. Consistency and accuracy of training movements were quantified offline by calculating the angular variance and compound acceleration of the first peak acceleration vector (table 1) (Stefan et al. 2008).
In the main experiment, while subjects perform the motor training, one of two interventions were delivered in separate sessions: (i) **Anodal tDCS** applied over the active M1 for a total of 30 minutes, and (ii) **Sham tDCS** applied in identical manner to the anodal session except that stimulation only lasted for 30secs at the beginning of training.

At the end of each intervention, 65 TMS-evoked thumb movement directions were determined again as done at baseline for approximately 11minutes (p1). Then, subjects rested for 10minutes and another 65 TMS pulses were applied (p2; figure 1).

At the end of each session, subjects reported their alertness, attention and perceived pain of tDCS using a self-scored visual analog scale (VAS) where 1 represented poorest attention, maximal fatigue and pain, and 7 maximal attention, least fatigue and pain (table 2) (Stefan et al. 2005).

**Controls**

*Duration of motor memories*
To assess the longevity of the effects observed with anodal tDCS, three subjects that took part in the main experiment were exposed to an additional block of TMS 50 minutes after the cessation of anodal tDCS and training (p3).

*Specificity of polarity effects on motor training*
To determine whether tDCS effects were polarity specific, three subjects who completed the main experiment underwent a 3rd session where training was performed under the influence of cathodal tDCS applied over the active M1 as done in the anodal session.

*Combination of training and tDCS*
To test whether stimulation alone could elicit changes in TMS-evoked movement directions, three additional subjects (2 females, mean age 24 ±3 years) were tested using
a similar experimental protocol however they received anodal tDCS for 30 minutes without performance of motor training.

**Data analysis**

*Training target zone (TTZ)*

We defined a training target zone (TTZ) as a window of ±20° centered on the mean direction of the performed training movements (Butefisch et al. 2000; Celnik et al. 2006; Stefan et al. 2008). The percentage of TMS-evoked thumb movements falling within the TTZ, the primary outcome measure, was calculated for pre, p1 and p2.

*TMS-evoked movement direction distance relative to training direction*

For each session we calculated the mean angular direction of the training movements. Then, the angular direction of each individual TMS-evoked movement was subtracted to this mean value (Classen et al. 1998). For example if the mean training direction was 320° and a movement in pre had an angular direction of 150° then the deviation from training would be 170° (figure 1). These values were averaged for each subject for pre, p1 and p2.

*Compound acceleration vector*

The mean magnitude of the first-peak acceleration in the extension/flexion direction during pre, p1 and p2 was calculated (Stefan et al. 2008). Since the principal training direction differed across subjects, we inverted the values so that all subjects had an extension movement within the pre block (Stefan et al. 2008).

*Corticomotor excitability*

We calculated the MEP amplitudes in muscles acting as either agonist or antagonist of the trained movement direction for each subject. Amplitudes were measured as peak-to-peak for each individual MEP and then averaged for pre, p1 and p2. The ratio between post and pre amplitudes for the agonist and antagonist muscles were calculated.
Statistical analysis

Separate repeated-measures ANOVAs (ANOVARM) were used for the percent of movements falling in TTZ, deviation from the training direction and compound acceleration with factors block (pre, p1, p2) and session (sham, anodal). ANOVARM was also used for the MEP ratio with factors muscle (agonist, antagonist), block (p1, p2) and session (sham, anodal). Measures of baseline corticomotor excitability (motor threshold, TMS stimulus intensity, MEPAGONIST and MEPANTAGONIST amplitudes), attention, fatigue, pain of tDCS and training movement kinematics (angular variance and compound acceleration) were analyzed with separate paired $t$-tests. When significant differences were found, post hoc analysis was performed using paired $t$-tests. Data are expressed as mean ± SEM, and effects were considered significant if $p \leq 0.05$.

Results

Summary

At baseline, TMS-evoked movements were consistent across sessions. Subjects were then trained in the opposite direction for 30 minutes (figure 2). Both, anodal and sham sessions showed training induced effects where the proportion of TMS-evoked movements reflected the practiced movement direction. However, this effect was more prominent in the anodal session immediately after the training (p1) and lasted longer (p2) relative to sham (figure 2).

[Figure 2]

Training parameters

Subject’s rating of attention, fatigue and pain were comparable across sessions (separate paired $t$-tests: $t_{(8)} \geq 0.9$, $p \leq 0.38$; table 1). For the sham session 5 out of 9 subjects believed they had received real stimulation, and 7 out of 9 subjects felt that way in the anodal session. Motor training kinematics measured as compound acceleration and angular variance did not differ across sessions (separate paired $t$-tests: $t_{(8)} \leq 1.2$, $p \geq 0.13$; table 1).
Preceding interventions, motor thresholds and MEP\textsubscript{ANTAGONIST} and MEP\textsubscript{AGONIST} amplitudes were comparable across sessions (separate paired \(t\)-tests: \(t_{(8)}\leq1.6, p\geq0.13\); table 1).

**Table 1**

**Effects of tDCS on motor memory formation**

ANOVA\textsubscript{RM} revealed a significant main effect for block (\(F_{(2,16)}=21.7, p<0.001\)), session (\(F_{(1,8)}=16, p=0.004\)), and block and session interaction (\(F_{(2,16)}=3.6, p=0.05\)) for the percent of movements falling within TTZ, the primary outcome measure. Paired \(t\)-tests showed that in both sham and anodal sessions there was a significant increase in the percentage of movements falling in the TTZ from pre to p1 (from 2±1\% to 12±4\% in sham, \(t_{(8)}=2.9, p=0.02\); and from 2±1\% to 17±4\% in anodal, \(t_{(8)}=7.4, p<0.001\); figure 3a). However, only in the anodal session was this effect significantly maintained at p2 (11±4\%, \(t_{(8)}=2.9, p=0.002\); figure 3a). In addition, in comparison to the sham session anodal had significantly more movements falling in the TTZ within p1 (\(t_{(8)}=2.6, p=0.015\)) and p2 (\(t_{(8)}=3.6, p=0.004\)).

**Figure 3**

**TMS-evoked movement direction distance relative to training direction**

ANOVA\textsubscript{RM} revealed a significant main effect for block (\(F_{(2,16)}=23.6, p<0.001\)), session (\(F_{(1,8)}=16.8, p=0.003\)), and block and session interaction (\(F_{(2,16)}=5.4, p=0.016\)). Post hoc paired \(t\)-tests showed that both sham and anodal sessions had a significant decrease in the relative angular distance from pre to p1 and p2 (sham: pre (146° ±4) to p1 (112° ±9) and p2 (128° ±10), \(t_{(8)}=4, p=0.002\), \(t_{(8)}=2.3, p=0.03\); and anodal: pre (150° ±4) to p1 (83° ±10) and p2 (98° ±14), \(t_{(8)}=8.4, p<0.001\), \(t_{(8)}=4.6, p=0.001\); figure 4a). However, the TMS-evoked movement directions in the anodal intervention was significantly closer to the training direction than sham during p1 (\(t_{(8)}=3.4, p=0.005\)) and p2 (\(t_{(8)}=2.7, p=0.02\); figure 4a).
Compound acceleration vector

ANOVA\textsubscript{RM} revealed a significant main effect for block (F(2,16)=10, p=0.001), session (F(1,8)=5.4, p=0.05) and block and session interaction (F(2,16)=6, p=0.01). Paired t-tests revealed that in both sham and anodal sessions there was a significant change in direction of compound acceleration from pre to p1 (from pre (0.36 m/s\textsuperscript{2} ±0.14) to p1 (-0.06 m/s\textsuperscript{2} ±0.05) in sham, t(8)=2.5, p=0.02; and from pre (0.42 m/s\textsuperscript{2} ±0.11) to p1 (-0.19 m/s\textsuperscript{2} ±0.1) in anodal, t(8)=3.6, p=0.003; figure 4b). However, only in the anodal session was this effect significantly maintained at p2 (-0.12 m/s\textsuperscript{2} ±0.08, t(8)=3, p=0.009; figure 4b). In addition, there was a significantly greater change in compound acceleration vector in the anodal session for p1 and p2 relative to sham (t(8)=2.4, p=0.02, t(8)=2.7, p=0.02 respectively; figure 4b).

Corticomuscular excitability

ANOVA\textsubscript{RM} revealed a significant main effect of session (F(1,8)=7.1, p=0.03), block (F(1,8)=20, p=0.002) and muscle (F(1,8)=41, p<0.001) when assessing MEP amplitudes. In addition, there were significant interactions between session and muscle (F(1,8)=10.2,p=0.01), and block and muscle (F(1,8)=7,p=0.03). Paired t-tests showed that within the sham session there was a significant difference between the increase of the MEP\textsubscript{AGONIST} ratio (post/pre) (1.4 ±0.15) in comparison to the MEP\textsubscript{ANTAGONIST} ratio (1.18 ±0.07) for p1 only (t(8)=2.4, p=0.02; figure 5b). Whereas in the anodal session, there was a significant difference at p1 (MEP\textsubscript{AGONIST} (1.8 ±0.2), MEP\textsubscript{ANTAGONIST} (1.14 ±0.16), t(8)=5.5, p=0.002) and p2 measures (MEP\textsubscript{AGONIST} (1.38 ±0.17), MEP\textsubscript{ANTAGONIST} (0.93 ±0.11), t(8)=2.8, p=0.04; figure 5b). In addition, relative to sham anodal session showed a significantly greater increase in the MEP\textsubscript{AGONIST} ratio for p1 (t(8)=2, p=0.05) and p2 (t(8)=2, p=0.05).
Control results

Only the anodal group showed a clear training effect during p2 suggesting that the motor memory had been retained for a longer period (approximately 30 mins). To test whether this effect was still present 50 minutes after the cessation of training, 3 subjects were given an additional block of TMS (p3, figure 3b). The percentage of movements falling in the TTZ were not significantly different between pre and p3 (2.2 ±2% and 3 ±1% respectively, paired t-test: \( t(2)=0.6, p=0.6 \)). Similarly, at p3 there were no significant differences in MEP\(_{AGONIST}\) and MEP\(_{ANTAGONIST}\) ratios (1.1 ±0.03mV and 0.9 ±0.08mV respectively, paired t-test: \( t(2)=2.1, p=0.16 \)) indicating a return to similar excitability to the pre measure.

To determine whether the previously described anodal tDCS effects were polarity specific, 3 subjects participated in a third session in which cathodal stimulation was applied over M1 during training. Here, the percentage of movements falling in the TTZ were not significantly different than sham stimulation in either p1 or p2 (cathodal: 10.4 ±0.6% and 2.1 ±0.6% respectively, ANOVA\(_{RM}\): session: \( F(1,2)=0.3, p=0.3 \); block: \( F(1,2)=53, p=0.001 \); session x block \( F(2,4)=0.6 p=0.6 \); figure 3c). MEP ratios were also statistically similar across sham and cathodal sessions for p1 (MEP\(_{AGONIST}\) (1.39 ±0.03), MEP\(_{ANTAGONIST}\) (1.1 ±0.09) and p2 measures (MEP\(_{AGONIST}\) (1.0 ±0.04), MEP\(_{ANTAGONIST}\) (1.0 ±0.1); ANOVA\(_{RM}\): session: \( F(1,2)=0.3, p=0.66 \); block: \( F(1,2)=23, p=0.04 \); muscle: \( F(1,2)=50, p=0.019 \); all interactions: \( F(1,2)\leq 5.6, p\geq 0.14 \)).

Finally, to determine whether anodal tDCS without training could elicit changes in TMS-evoked movement directions we studied an additional group of 3 subjects. In this group there was no significant difference in the percentage of movements falling in the TTZ between pre (1 ±0.7%) and p1 (1 ±0.5%, paired t-test: \( t(2)=0.5, p=0.7 \); figure 3d). Similarly, there was no significant difference between MEP\(_{AGONIST}\) and MEP\(_{ANTAGONIST}\) muscle ratios (1.09 ±0.02 and 1.1 ±0.07 respectively, paired t-test: \( t(2)=0.3, p=0.8 \)).
Discussion

The main finding of the present study was that anodal transcranial direct current stimulation (tDCS) over the primary motor cortex, engaged in generating the training movements, enhanced the encoding and retention of motor memories. This effect was reflected by (1) changes in all kinematic measures (movements falling in the training zone, angular differences and compound muscle accelerations), (2) longer lasting effects relative to training alone, (3) the required association of training and stimulation, and (4) the polarity specificity.

Previous studies have shown that performing simple motor training results in the formation of motor memories containing kinematic details of the practiced motions (Butefisch et al. 2002, 2004; Celnik et al. 2006; Classen et al. 1998; Floel et al. 2005 a,b; Stefan et al. 2008). Consistent with these, we found that motor training with sham tDCS increased the probability of TMS-evoked movements falling in the TTZ, decreased the net TMS-evoked movement direction distance relative to the training direction, and changed toward the direction of the practiced movements the net acceleration of all TMS-evoked thumb movements. However, when the same group of participants performed the motor training under the influence of anodal tDCS they experienced larger gains in all measures in p1, and maintained the effects into p2. These findings appear to be polarity specific, since training during cathodal tDCS resulted in similar effects as training with sham, and required the association of physical practice with anodal stimulation, as tDCS alone did not result in any kinematic change.

Since subjects performing the training combined with anodal tDCS have persistence of the effects 30mins after the completion of training (p2), longer motor memory retention, we decided to investigate the longevity of the motor memory formed. With this aim, we retested a subgroup of participants 50 minutes after the cessation of training and stimulation (p3). Across all measures, performance returned to pre-training values, indicating that any changes within the motor cortex representation had reverted back to baseline. This type of motor memory encoding enhancement has been previously
observed with D-Amphetamine (Butefisch et al. 2001, Sawaki et al. 2002a), hebbian-type TMS stimulation during training (Butefisch et al. 2004) and congruent action observation (Celnik et al. 2006; Stefan et al. 2008). Of note, as ratings of attention, fatigue and pain of the tDCS were similar across sessions, it is unlikely that these results could directly be explained by unspecific psychological differences across sessions.

Similar to previous studies, we found that training resulted in a specific increase in the excitability of the training muscles (Butefisch et al. 2004; Celnik et al. 2006; Classen et al. 1998). Interestingly, anodal tDCS increased the excitability of the muscle agonist to the training without a similar change in the antagonist muscle, suggesting that tDCS had a training-specific effect rather than a global increase in corticomotor excitability. These results together with the findings that the no training plus tDCS control group had minimal changes in the excitability of either muscle, suggest that the effect of tDCS on cortical excitability are more prominent on neuronal populations engaged in motor actions.

Cathodal tDCS, although known to reduce the excitability of the motor cortex at rest (Purpura and McMurtry, 1965; Nitsche et al. 2003a), in the present study did not influence corticomotor excitability relative to sham. It is possible that this lack of effect was due to the performance of motor training, which elicits increased excitability (Butefisch et al. 2002, 2004; Celnik et al. 2006; Classen et al. 1998; Floel et al. 2005 a,b; Stefan et al. 2008) thus overriding or masking the effect of cathodal tDCS.

The mechanism behind the current enhancement of motor memory encoding through anodal tDCS is unknown. However, this form of motor memory encoding is influenced by NMDA-receptor function (Butefisch et al. 2000), and it has been indirectly shown that within humans the mechanisms underlying the effect of tDCS involves the modulation of NMDA-receptors (Nitsche et al. 2003d). Therefore, it is plausible that the observed results are due to anodal tDCS increasing the efficacy of NMDA-receptor function, possibly through an increase in intracellular calcium concentration (Bennett, 2000).
A previous study by Rosenkranz et al. (2000) explored the effect of tDCS during an identical paradigm to the present study by only applying tDCS during the last 10 minutes of training. The authors found that both anodal and cathodal tDCS had a negative effect on the formation and retention of a motor memory. We believe that the stark difference in results may be due to the differing tDCS protocols. Work by Iezzi et al. (2008) and Siebner et al. (2004) have shown that a TMS protocol can either be facilitatory or inhibitory depending on the prior state of the system. It is possible that applying tDCS after 20 minutes of training has a different effect to tDCS at the onset of training due to the pre-conditioning of the motor cortex resulting from training. Alternatively, it is possible that the lack of findings in Rosenkranz investigation was due to shorter stimulation period.

Non-invasive cortical stimulation with tDCS elicits behavioral gains during implicit motor-learning (Nitsche et al. 2003d), visuo-motor processing (Antal et al. 2004, Reis et al. 2009) and probabilistic learning (Kincses et al. 2004) in healthy volunteers. In addition, it has been shown to improve motor performance in stroke (Hummel et al. 2005) and Parkinson disease patients (Boggio et al. 2006). More recently, Reis et al, have reported that tDCS in association to training enhances learning of a novel skill by modulation of short-term retention (Reis et al. 2009). The present study supports these observations and suggests that anodal tDCS enhances both the process of encoding and retention of simple motor memories. In addition, we can rule out changes in memory recall mechanisms since the behavioral measures obtained with the TMS paradigm used here do not require voluntary engagement of recall processes.

One of the initial steps in the development of more complex skills is the encoding of motor memories (Classen et al. 1998, Molina-Luna et al. 2008). It has been suggested that the motor cortex may encode the memory trace of a skill (Monfils et al. 2005) as changes in corticomotor organization occur during training (Karni et al. 1995; Kleim et al. 2004; Nudo et al. 1996a,b,c), and skill learning is associated with an enlargement of representations of trained body parts (Pascual-Leone et al. 1995). Therefore this study, showing that anodal tDCS over M1 enhances the motor memory formed after simple
repetitive practice, may provide evidence as to how this form of stimulation results in the behavioral gains observed in previous studies (Antal et al. 2004; Kincses et al. 2004; Nitsche et al. 2003d; Reis et al. 2009).

In summary, we showed that anodal tDCS enhances the effects of simple repetitive training on the formation and retention of motor memories. This effect was polarity-specific, relied on the interaction between motor training and tDCS, and lasted longer than performance of training without stimulation. The observed changes in the motor cortex representation may play an important role in the acquisition of more complex skills. The current results provide rational to the possible mechanism that may underlie the behavioral gains observed with the application of tDCS in previous studies. It also supports the view that such noninvasive cortical stimulation can be a useful tool in the rehabilitation of patients with brain damage.
Grants

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Table legends

Table 1

Baseline measures.

Psychological measures. Values (mean ± SEM) depict subject’s choice in a visual analog scale where 1 represents poorest attention, maximal fatigue and pain, and 7 maximal attention, least fatigue and pain. Motor training kinematics. Units (mean ± SEM) are m/s² for compound acceleration and degrees for angular variance. Measures of corticomotor excitability preceding interventions. Units (mean ± SEM) are % of stimulator’s output for motor threshold, and mV for MEP amplitudes elicited in muscles acting as antagonist and agonist to the trained thumb movements. P and t values originate from paired t-tests performed on the sham and anodal sessions.
Figure legends

**Figure 1. Schematic representation of the main experiment setup.** (a) *Pre.* TMS-evoked movement directions were derived from the first-peak acceleration in the two major axes of the movement (extension/flexion and abduction/adduction) measured by an accelerometer mounted on the proximal phalanx of the thumb. Black arrows indicate the direction of individual TMS-evoked thumb movements (in this case extension and abduction). (b) *Motor training.* Pre was followed in two separate sessions randomly ordered by thirty minutes of motor training with either sham or anodal tDCS being applied over the left M1. Voluntary thumb movements were performed in a direction opposite to the baseline TMS-evoked movement direction (in this case: flexion and adduction). (c) *Post 1 (p1).* The direction of TMS-evoked thumb movements were determined as previously measured in pre. (d) *Post 2 (p2).* The subjects rested for ten minutes and the direction of TMS-evoked thumb movements were again determined.

**Figure 2. Intervention-dependent changes in TMS-evoked thumb movement directions in a representative subject.** Each line represents the first-peak acceleration direction vector of a single thumb movement. The pre block is characterized by predominantly extension/adduction movement directions in both sessions. (a) After motor training with anodal tDCS the direction of TMS-evoked thumb movements (p1) changed to a direction similar to training (flexion/abduction). This was partially maintained in p2. (b) After training with sham tDCS the direction of TMS-evoked thumb movements within post1 did not have such a dramatic change with only a small proportion of movements moving in a direction similar to motor training. For p2 all movements were in a direction similar to the pre block.

**Figure 3. Percentage of movements falling in TTZ (pTTZ).** (a) With anodal tDCS applied during training there was a significant increase in movements falling within the TTZ during p1 and p2. Asterisks denote $p \leq 0.02$. (b) 50 minutes after the cessation of anodal tDCS (p3) TMS-evoked movement directions returned to pre-motor training values (n=3). (c) TMS-evoked movement directions were similar between sham and cathodal
sessions at p1 and p2 times (n=3). (d) Application of anodal tDCS for 30 minutes without motor training did not elicit changes in pTTZ. Data are means ±SEM.

**Figure 4.** *Angular distance and compound acceleration of TMS-evoked movements.* (a) Angular distance of TMS-evoked movements relative to training during pre, p1 and p2. In comparison to sham, anodal tDCS led to a greater reduction in the angular distance between training movements and those evoked by TMS at p1 and p2 times. Please note that at pre, both sessions have similar angular difference from the trained direction. (b) Average compound acceleration during pre, p1 and p2. Anodal tDCS led to a greater change in direction of compound acceleration during p1 and p2 relative to sham. Asterisks, $p \leq 0.03$. Data are means ±SEM.

**Figure 5.** *Corticomotor excitability, measured by the MEP amplitude for the agonist and antagonist muscle involved in motor training.* (a) Changes in the MEP amplitude recorded from the training antagonist (closed squares) and agonist (open diamonds) muscles. MEP amplitudes increased with both sham and anodal tDCS. (b) MEP amplitude ratio (post/pre) significantly increased in the MEP$_{AGONIST}$ muscle in comparison to the MEP$_{ANTAGONIST}$ during p1 for both sham and anodal tDCS. For anodal tDCS this difference was maintained during p2. For both p1 and p2, anodal tDCS resulted in a greater increase in the MEP$_{AGONIST}$ ratio. Asterisks, $p \leq 0.05$. Data are means ±SEM.
Enhancement of motor memories

References


<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham</th>
<th>Anodal</th>
<th>Paired t-test: sham x anodal</th>
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<td><strong>Psychological measures</strong></td>
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<td>Attention (VAS)</td>
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<td>Fatigue (VAS)</td>
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<td><strong>Motor Training Kinematics</strong></td>
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<td>Compound acceleration (m/s²)</td>
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<td>1.06 ± 0.23</td>
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<td>Angular variance (deg)</td>
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<td>21 ± 3</td>
<td>p=0.26 t=1.2</td>
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<td><strong>Measures of Corticomotor Excitability</strong></td>
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<tr>
<td>Motor threshold (agonist muscle) %</td>
<td>45 ± 2</td>
<td>43 ± 2</td>
<td>p=0.13 t=1.6</td>
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<tr>
<td>MEPantagonist (mV)</td>
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<td>3.1 ± 0.6</td>
<td>p=0.9 t=0.14</td>
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<td>MEPagonist (mV)</td>
<td>1.3 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>p=0.9 t=0.05</td>
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Table 1
Figure 1

**Pre**
65 trials

**Motor training**
30 mins

**p1**
65 trials

**rest**
10 mins

**p2**
65 trials

**a.**
Accelerometer

**b.**
Anodal/sham tDCS

**c.**
Flexion

**d.**
Abduction

---

**Extension**

**Adduction**

**Flexion**
Figure 2

pre TMS-evoked mvts

motor training

p1 TMS-evoked mvts

p2 TMS-evoked mvts

a

Extension

Adduction

Flexion

2 m/s²

b

anodal tDCS

sham tDCS
Figure 3
Figure 4

(a) TMS-evoked movement direction angles relative to training (degrees)

(b) Compound acceleration (m/s²)
Figure 5

a. MEP amplitude (mV)

b. MEP ratio (post/pre)