

Cerebellar Transcranial Direct Current Stimulation Impairs the Practice-dependent Proficiency Increase in Working Memory

R. Ferrucci, S. Marceglia, M. Vergari, F. Cogiamanian,
S. Mrakic-Spota, F. Mameli, S. Zago, S. Barbieri, and A. Priori

Abstract

■ How the cerebellum is involved in the practice and proficiency of non-motor functions is still unclear. We tested whether transcranial direct current stimulation (tDCS) over the cerebellum (cerebellar tDCS) induces after-effects on the practice-dependent increase in the proficiency of a working memory (WM) task (Sternberg test) in 13 healthy subjects. We also assessed the effects of cerebellar tDCS on visual evoked potentials (VEPs) in four subjects and compared the effects of cerebellar tDCS on the Sternberg test with those elicited by tDCS delivered over the prefrontal cortex in five subjects. Our experiments

showed that anodal or cathodal tDCS over the cerebellum impaired the practice-dependent improvement in the reaction times in a WM task. Because tDCS delivered over the prefrontal cortex induced an immediate change in the WM task but left the practice-dependent proficiency unchanged, the effects of cerebellar tDCS are structure-specific. Cerebellar tDCS left VEPs unaffected, its effect on the Sternberg task therefore seems unlikely to arise from visual system involvement. In conclusion, tDCS over the cerebellum specifically impairs the practice-dependent proficiency increase in verbal WM. ■

INTRODUCTION

Although the role of the cerebellum in motor practice is relatively well established (Van Mier & Petersen, 2002), how and whether the cerebellum is involved in the practice of nonmotor functions is still controversial.

Working memory (WM) temporarily stores an active representation of information. WM supports cognitive processes by providing an interface between perception, long-term memory, and behavioral responses (Baddeley, 1992). Although day-to-day experience shows that WM improves with practice (Kirschen, Chen, Schraedley-Desmond, & Desmond, 2005), the mechanisms underlying its practice-dependent proficiency increase remain unclear. Some evidence suggests cerebellar involvement in WM and its practice-dependent increase (Kirschen et al., 2005). The primate cerebellum has known cognitive and psychic functions (Allen, Muller, & Courchesne, 2004; Fiez, 1996; Middleton & Strick, 1994). For example, Kirschen et al. (2005) have shown that the practice-dependent increase in the efficiency of a verbal WM (VWM) task correlates with the increase in cerebro-cerebellar network activation, thus suggesting that cerebellar changes underlie the practice-dependent improvement in VWM. Whether the practice-dependent increase in cerebellar activation is primarily

involved in the increased task proficiency, or is just an epiphenomenon of increased activity in the cerebral cortex, nonetheless remains unclear.

Transcranial direct current stimulation (tDCS) is a recent noninvasive technique for focal modulation of brain activity (Wassermann & Grafman, 2005; Paulus, 2004; Priori, 2003; Nitsche & Paulus, 2000; Priori, Berardelli, Rona, Accornero, & Manfredi, 1998). Weak electrical direct current (<1.5 mA) applied over the scalp induces prolonged changes in brain excitability that persist long after current offset. Several studies, investigating changes in the motor evoked potentials (MEPs) elicited by transcranial magnetic stimulation (TMS), have shown that tDCS delivered over the motor cortex modulates corticospinal output for more than an hour after the current offset (Ardolino, Bossi, Barbieri, & Priori, 2005; Priori, 2003). tDCS delivered over the cerebral cortex also elicits cognitive changes (Priori et al., 2008; Boggio et al., 2006; Fregni et al., 2005; Marshall, Molle, Siebner, & Born, 2005). The after-effects of brain polarization arise from both synaptic and nonsynaptic functional changes (Ardolino et al., 2005; Liebetanz, Nitsche, Tergau, & Paulus, 2002). Although the primate cerebellum has a wide cortical surface, no study has so far assessed whether—by analogy with tDCS over the cerebral cortex—tDCS over the cerebellum (cerebellar tDCS) can elicit prolonged after-effects on its functions. The importance of this information lies in the possible therapeutic application of cerebellar cortex modulation

in conditions involving cerebellar dysfunction, such as autism (Allen et al., 2004).

In this study, we investigated whether the human cerebellum is primarily responsible for the practice-dependent increase in proficiency in a VWM paradigm. To do so, in healthy volunteers, before tDCS over the cerebellum began, at 5 and at 35 min after it ended, we tested VWM with a repeated modified Sternberg paradigm (Sternberg, 1966). Clinical (Desmond et al., 2003), neuroimaging (Chen & Desmond, 2005), and TMS (Desmond, Chen, & Shieh, 2005) studies demonstrate cerebellar involvement in the Sternberg task. Findings from studies on human lesions suggest that cerebellar structures contribute to various forms of learning (Gordon, 2007; Torriero et al., 2007; Fiez, Petersen, Cheney, & Raichle, 1992). In additional control experiments, we then assessed the possible spread of cerebellar tDCS to the occipital cortex by recording visual evoked potentials (VEPs) and investigated the specificity of VWM changes by delivering tDCS over the prefrontal cortex.

METHODS

Subjects

Seventeen healthy right-handed volunteers (aged 19–32 years) participated in the study. Several subjects participated in more than one experiment. All participants gave their informed consent and the procedures had the approval of the hospital ethical committee. The experimental procedure was in accordance with the Declaration of Helsinki. All participants received a neurological examination before and after tDCS end using paper-and-pencil tests of motor-graphic (signature, Archimed spiral, and horizontal lines test). At the end of every tDCS session and within the 24 hours poststimulation, participants completed an ad hoc questionnaire, to test for possible adverse effects (including headache, nausea, and impaired balance).

Transcranial Direct Current Stimulation

tDCS was bilaterally delivered by two electrical constant direct current stimulators each connected to a pair of sponge electrodes. Two sites of tDCS were used: one over the cerebellum and the other over the prefrontal cortex. For cerebellar tDCS, electrodes were placed on the scalp over the cerebellum (2 cm under the inion, 1 cm posterior to the mastoid process) and the other one over the right deltoid muscle; for prefrontal cortex tDCS, electrodes were placed one over the prefrontal cortex (between Fp1 and F3 for the left side and between Fp2 and F4 for the right side) and the other over the right deltoid muscle. To avoid confounding biases arising from two electrodes with opposite polarities over the scalp, we used a noncephalic reference electrode for tDCS (Priori et al., 2008; Cogiamanian, Marceglia,

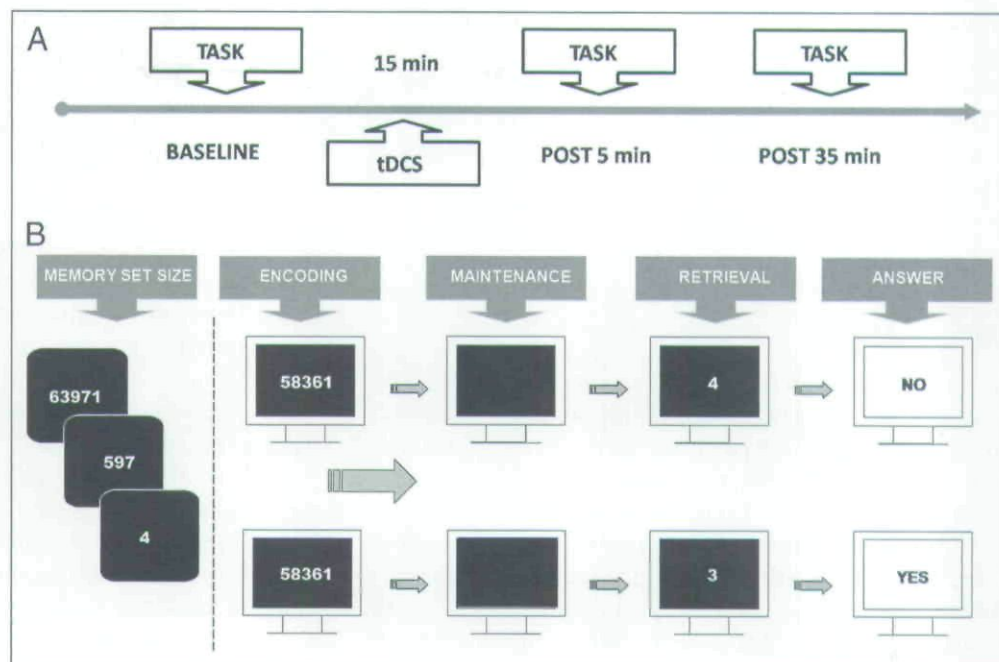
Ardolino, Barbieri, & Priori, 2007). The electrodes used for tDCS were thick (0.3 cm), elliptical saline-soaked synthetic sponges (scalp electrode 21 cm²; deltoid electrode 64 cm²). The wide electrode surface avoided the possible harmful effects of high current density. The stimulating current was an anodal or cathodal direct current at 2 mA intensity delivered for 15 min over the cerebellum or the prefrontal cortex bilaterally. When stimulation began, subjects felt the current at both electrodes as a mild itching sensation that disappeared after a few seconds thereafter leaving tDCS unperceived. For sham tDCS, electrodes were placed as for real stimulation but the stimulator was turned off after 10 to 15 sec. The subjects therefore felt the initial itching sensation when stimulation began but thereafter received no current. Subjects were tested before tDCS began and at 5 and 35 min after it ended (Figure 1A). During the cerebellar and the prefrontal tDCS sessions, subjects were studied three times, once for each stimulation type (anodal, cathodal, sham). One month elapsed between cerebellar and prefrontal tDCS sessions, and at least 1 week between anodal, cathodal, and sham stimulation. Stimulations were applied in random order (for instance, first week anodal, second week cathodal, third week sham) balanced across subjects.

To guarantee safety, we applied a current at a density of 0.095 mA/cm² and delivered a total charge of 0.086 C/cm². These criteria, in agreement with Nitsche et al. (2003), are far below the threshold for tissue damage (Boggio et al., 2006). Because the resistivity of the skull is higher than that of the scalp, most of the current delivered by transcranial stimulation gets shunted through the scalp (Miranda, Lomarev, & Hallett, 2006). The amount of current density in the scalp tends to decrease with the distance between electrodes (Nathan, Sinha, Gordon, Lesser, & Thakor, 1993). The current density generated in the cortex by the stimulation decreases rapidly with depth, that is, it decreases by one order of magnitude in 8 mm (Nathan et al., 1993). It is therefore unlikely that current flow in the brainstem and structures other than the cerebellar or cerebral cortex below the stimulating electrode could influence the present experiment. Also, the noncephalic reference electrode montage is reported to yield highly reproducible data during and after polarization without inducing effects related to brainstem activation (Accornero, Li Voti, La Riccia, & Gregori, 2007). Finally, intraoperative results demonstrate that, for eliciting a MEP by directly stimulating the human brainstem, a current density of about 2 to 9 mA/cm² is required (Cedzich, Pechstein, Schramm, & Schafer, 1998).

Working Memory Task

A modified Sternberg VWM task was administered to each participant. Stimuli were presented on a PC screen using

Figure 1. Experimental protocol. (A) Schematic diagram of the experimental design during a single session. (B) The task sequence for present and absent answers. On the left, the three memory set sizes (1, 3, and 5). The top gray horizontal line shows the different phases of the task (encoding, maintenance, and retrieval). The upper panel represents the sequence for absent answers and the lower panel the sequence for present answers.



the Wadsworth CogLab software (Wadsworth Publishing, Belmont, CA, USA). Subjects were shown a short list of numbers simultaneously (one to five) and asked to memorize them. After memorizing them, subjects were shown a probe number, either one of the numbers in the list (present answer) or a new one (absent answer). When the VWM task began, a warning signal appeared on the center of the screen and, 1 sec later, a memory set consisting of one, three, or five numbers appeared on the screen at an interstimulus interval of 1.2, 3.6, or 6 sec, respectively (encoding phase). When the memory trials ended, the memory set disappeared and, 2 sec later (maintenance phase), a probe item appeared (retrieval phase) and remained on the screen until the subject answered. The subjects were asked to answer whether the probe was among the targets by pressing one of two keys on the keyboard one for present and one for absent (Figure 1B). To reduce the spatial stimulus-response effect on the reaction time (RT), the position of the probe in the presented sequence was counterbalanced over all possible positions. We balanced the subjects for the type of response (present or absent) with the right and left hand.

According to task logic, RTs reflect the time spent searching in short-term memory (STM) to determine whether the probe number is part of the list. Subjects received a feedback for right and wrong answers but no feedback about RT. Experimental sessions consisted of 60 trials, 10 each for present and absent conditions with memory set size 1, 3, and 5. RTs and accuracy were computed. Subjects were instructed to be fast and accurate in their responses and were all motivated to do well. To familiarize subjects with the VWM task, a session of practice trials was run. Subjects were included

in the study only if their baseline RTs was between 480 and 1300 msec.

Three different types of stimulation (anodal, cathodal, and sham) were tested, in random order, in three separate experimental sessions at intervals of at least 1 week. In each session, the task was administered three times: under no-stimulation (baseline), at 5 min after tDCS ended, and at 35 min after tDCS ended. Of the 17 participants, 13 subjects underwent the VWM task before and after tDCS over the cerebellum. Of these 13, 5 (1 man and 4 women) underwent the same task before and after tDCS over the prefrontal cortex. At least 1 month elapsed between the cerebellar session and the prefrontal session. The other four subjects underwent only the following VEP protocol.

Visual Evoked Potentials

Because tDCS over the occipital cortex elicits significant changes in VEPs (Accornero et al., 2007), to exclude the possible spread of cerebellar tDCS to the occipital cortex, we measured VEPs in four subjects before, 5 min after, and 35 min after anodal and cathodal tDCS over the cerebellum ended. tDCS was delivered with the same procedure used for the VWM task. In all subjects, VEPs were elicited with black-and-white pattern-reversal checkerboards (2 cycles/deg; Viking IV P, NIC 1015 monitor, Nicolet Biomedical), and reversed at two spatial frequencies (15° and 30°). Signals were recorded through nonpolarizable Ag/AgCl surface electrodes with the reference electrode placed on the vertex and the recording electrode 1 cm above the inion. The acquisition system automatically rejected artifacts

and bandpassed signals between 0.5 and 100 Hz. VEPs were recorded by averaging 50 traces. Because the widest amplitude and most stable component in response to this kind of stimulation is P100, we evaluated this component alone (Accornero et al., 2007). We measured P100 amplitude (P100–N75 and P100–N145) and its peak latency.

Data Analysis

In the VWM task, RTs and accuracy were collected and used as dependent variables. RTs and accuracy data from the cerebellar tDCS experiment were tested with a preliminary four-way repeated measures analysis of variance (ANOVA) (STATISTICA 5.5, StatSoft) with main factors “memory task,” three levels (1, 3, 5); “answer type,” two levels (absent and present); “stimulation,” three levels (anodal, cathodal, and sham); “time,” three levels (baseline, 5 min, and 35 min); and significance level at $p < .05$. Tukey honest significant difference post hoc test was used to compare variables in subgroups ($p < .05$). The results of this preliminary analysis were used to adapt ANOVA factor levels. In addition, a nonparametric Wilcoxon signed-ranked test was used to compare RT percentage changes after anodal and cathodal stimulation. RT percentage changes were calculated as $(35 \text{ min RT} - \text{baseline RT})/(\text{baseline RT})$. The data from the prefrontal tDCS experiment were analyzed with a similar approach using a preliminary four-way repeated measures ANOVA with “memory task,” three levels (1, 3, 5); “answer type,” two levels (absent and present); “stimulation,” three levels (anodal, cathodal, and sham); and “time,” three levels (baseline, 5 min, and 35 min) as main factors. The results of this preliminary analysis were used to adapt ANOVA factor levels. As previously performed on data collected during cerebellar tDCS experiments, we applied a nonparametric Wilcoxon signed-ranked test on RT percentage changes $(5 \text{ min RT} - \text{baseline RT})/(\text{baseline RT})$.

To compare data from the cerebellar and the prefrontal sessions, a two-way ANOVA with “stimulation site” (independent measures) and “stimulation type” (repeated measures) as main factors was run on RT percentage changes ($p < .05$).

To evaluate VWM task learning rates over time, we also calculated the linear regression analysis (GraphPad Prism software, GraphPad Software) on RTs in time. In particular, we evaluated the normal VWM learning task through a regression analysis of baseline RT evolution in time. For each subject, the regression slope was estimated from baseline RTs obtained in the six consecutive experiments (3 stimulations in the cerebellar session and 3 in the prefrontal session).

The same regression analysis was also run on RTs before tDCS began, and at 5 and 35 min after tDCS ended in each experimental session and for each sub-

ject. The learning rate of the Sternberg paradigm was represented by the slope of the estimated lines:

$$y = at + b \quad (1)$$

where y is the dependent variable (RT), t is time, a is the slope, and b is the y -intercept of the line.

In a previous work, Sternberg (1969) found a linear fitting between RT and memory set size. The slope of the RT/memory set size function provides a measure of the time taken to compare the test stimulus with the representation of that stimulus in memory (serial comparison or scanning) that is independent of primary perceptual or motor system functioning. The slope of the RT function therefore provides an estimate of speed of cognitive processing (Archibald & Fisk, 2000). Conversely, the zero-intercept (or y -axis intercept) of this function provides an estimate of how long it takes to encode the test stimulus, make a binary decision, and organize a response, and is therefore influenced by disruption in perceptual processing or motor functioning or both. Thus, we calculated the linear function RT/memory set size before and after cerebellar tDCS and before and after prefrontal tDCS. The effect of tDCS was assessed in terms of slope percentage changes $(\text{after tDCS} - \text{before tDCS})/(\text{before tDCS})$. Percentage changes after cerebellar tDCS and prefrontal tDCS were compared with percentage changes after sham in terms of standard deviations from the mean sham slope. Values $< 1SD$ were considered as mild improvement (Archibald & Fisk, 2000).

The effect of stimulation on VEPs was tested through a three-way ANOVA with repeated measures main factors: “stimulation,” two levels (anodal and cathodal); “time,” three levels (baseline, 5 min, and 35 min); and “frequency,” two levels (30° and 15°). Values are expressed as mean \pm standard deviation.

RESULTS

Of the 13 subjects tested, 2 reported experiencing mild headache after cathodal and sham tDCS over the cerebellum. None had ataxia, incoordination, or tremor.

tDCS over the Cerebellum

Whereas the type of cerebellar tDCS applied (anodal, cathodal, and sham) had no influence on data for task accuracy, it significantly influenced RTs. Because the preliminary four-way ANOVA, performed on the entire dataset, showed that tDCS of the cerebellum had no significant effect on RTs measured at 5 min (Stimulation \times Time, $p = .12$; post hoc test: anodal baseline vs. 5 min, $p = .99$; cathodal baseline vs. 5 min, $p = .97$), the subsequent analysis therefore focused on tDCS-induced

changes at 35 min (i.e., the ANOVA main factor "time" levels became baseline and 35 min). Also, because anodal and cathodal tDCS had the same effect on RT changes at 35 min (anodal RT change: -0.04 ± 0.19 ; cathodal RT change: -0.01 ± 0.19 ; $p = .2$, Wilcoxon's signed rank test, Figure 2A), in subsequent analysis, the data for both types of tDCS were pooled: For each subject, RTs for anodal and cathodal stimulation were averaged and compared with the sham (i.e., the ANOVA main factor "stimulation" level became stimulation and sham).

ANOVA showed a significant effect of the main factors task ($p < .000001$) and answer type ($p < .0005$): As task complexity increased, RTs significantly lengthened (Set size 1: 711.5 ± 183.1 msec; Set size 3: 821.1 ± 189.1 msec; Set size 5: 926.0 ± 223.0 msec) and RTs were significantly longer for absent answers than for present answers (854.8 ± 203.5 vs. 785.0 ± 179.8 msec). The main factor time had a significant effect on RTs ($p = .008$). More important, it significantly interacted with the factor stimulation (Stimulation \times Time: $p = .03$). Post hoc analysis showed that, after sham, RTs decreased significantly from baseline (baseline: 849.5 ± 236.8 msec; 35 min: 730.7 ± 179.4 msec, $p = .001$), whereas after tDCS they remained unchanged (baseline: 850.3 ± 215.0 msec; 35 min: 811.0 ± 208.8 msec, $p = .36$, Figure 3). Also, whereas baseline RTs were similar for sham and tDCS, RTs measured at 35 min after tDCS ended differed under the two conditions ($p = .02$, Figure 3).

Finally, ANOVA disclosed a significant three-factor interaction between answer type, stimulation, and time (Answer type \times Stimulation \times Time: $p = .04$): RTs for both present and absent answers decreased significantly after sham (absent – baseline: 888.8 ± 215.0 msec; 35 min: 760.4 ± 179.4 msec, $p = .00031$; present – baseline: 810.2 ± 190.3 msec; 35 min: 701.0 ± 133.5 msec,

$p = .0009$). In contrast, RTs for present answers decreased significantly after tDCS (baseline: 828.7 ± 169.1 msec; 35 min: 760.2 ± 156.3 msec, $p = .02$), whereas RTs for absent answers remained unchanged (baseline: 872.0 ± 195.2 msec; 35 min: 861.8 ± 179.0 msec, $p = .99$). Also, RTs for absent answers after tDCS were significantly longer than RTs for absent answers after sham ($p = .0015$) and RTs for present answers after tDCS ($p = .0014$). The nonparametric analysis of RT changes confirmed the significant difference between sham and real cerebellar tDCS ($p = .016$).

In summary, anodal and cathodal tDCS over the cerebellum blocked the RT decrease induced by repeating the task without tDCS. This effect became evident 35 min after cerebellar tDCS ended.

tDCS over the Prefrontal Cortex

Prefrontal tDCS left accuracy unchanged but had a significant effect on RTs. Because the prefrontal tDCS session followed the cerebellar tDCS session, mean baseline RTs in the prefrontal session were significantly shorter than those in the cerebellar session (849.91 ± 184.5 msec vs. 677.74 ± 97.69 msec, $p = .0086$). The preliminary four-way ANOVA showed that the shortening effect remained evident at 5 min (main factor "time", $p = .024$). In particular, post hoc analysis disclosed that RTs measured at 5 and 35 min after tDCS ended differed significantly from baseline RTs (baseline vs. 5 min, $p = .03$; baseline vs. 35 min, $p = .049$), but did not differ from each other at the two time points (5 min vs. 35 min, $p = .94$). We therefore specifically analyzed the effect of tDCS after 5 min. ANOVA confirmed the effect of the main factors "task" ($p = .00003$) and "answer type" ($p = .029$) on RTs. Wilcoxon nonparametric test for paired data was used to compare RT changes after anodal and cathodal

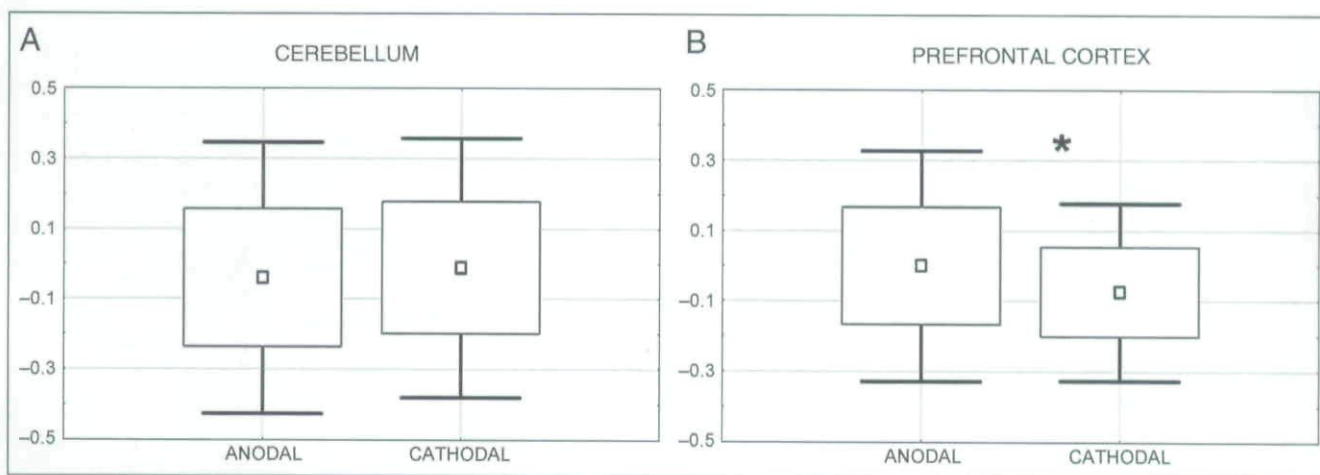


Figure 2. Effects of anodal and cathodal tDCS on WM task RTs after 35 min of cerebellar tDCS and after 5 min of prefrontal tDCS. The box-and-whisker plots are composed of the central small square representing the mean, the large square representing the mean \pm standard deviation (SD), and the bars representing the mean \pm 1.96 SD (95% CI). Data are variations in RTs [expressed as (RT after – RT before)/(RT before)] after stimulation of the cerebellum (A) and of the prefrontal cortex (B). Note that whereas anodal and cathodal tDCS have the same effect on RTs after cerebellar stimulation, cathodal tDCS produces shorter RTs than anodal tDCS over the prefrontal cortex.

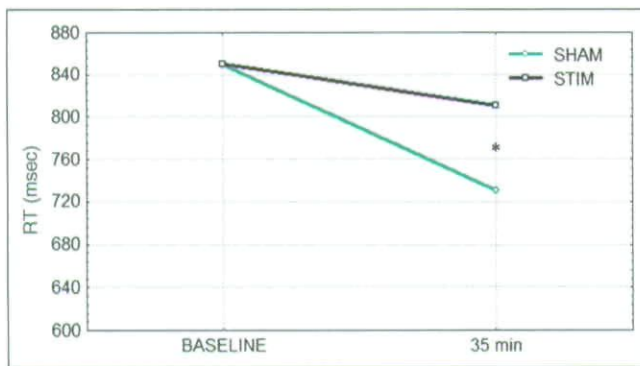


Figure 3. Mean RTs after sham and tDCS over the cerebellum. Interaction between the factor stimulation and the factor time. Colored lines represent RTs for sham (green) and stimulation (black) at baseline and after 35 min of tDCS ended. The effect was significant ($*p = .03$). Note that whereas after sham stimulation RTs significantly decrease, after tDCS RTs remain unchanged.

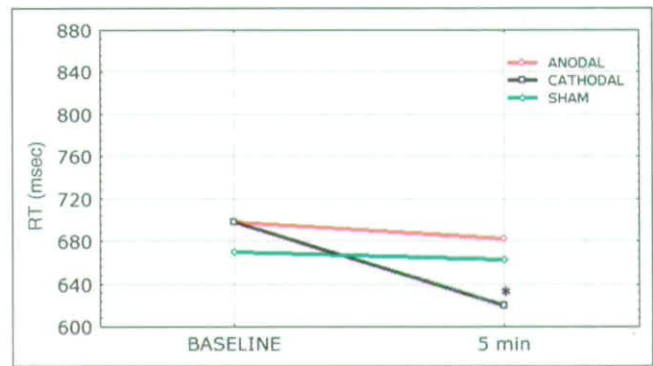


Figure 4. Mean RTs after anodal, cathodal, and sham tDCS over the prefrontal cortex. Interaction between factors stimulation type and time. Colored lines represent RTs for memory anodal (red), cathodal (black), and sham (green) stimulation at baseline and at 5 min after tDCS ended. Note that whereas anodal and sham tDCS leave RTs unchanged, cathodal tDCS significantly shortens RTs. $*p < .05$.

stimulation (5 min). Anodal and cathodal tDCS over the prefrontal cortex had a significantly different effect on RTs (anodal: -0.01 ± 0.12 msec; cathodal: -0.10 ± 0.09 msec, $p = .043$, Figure 2B). ANOVA performed on RTs at baseline and at 5 min showed a significant interaction between the factor "stimulation" and the factor "time" (Stimulation \times Time, $p = .013$, Figure 4). Post hoc

analysis showed that cathodal tDCS decreased RTs (baseline: 698.33 ± 142.66 msec; 5 min: 619.5 ± 113.54 msec, $p = .0041$, Figure 4).

The ANOVA comparing the effect of tDCS over the prefrontal cortex with that over the cerebellum disclosed a significant interaction between the factors "stimulation site" and "stimulation type" on percentage

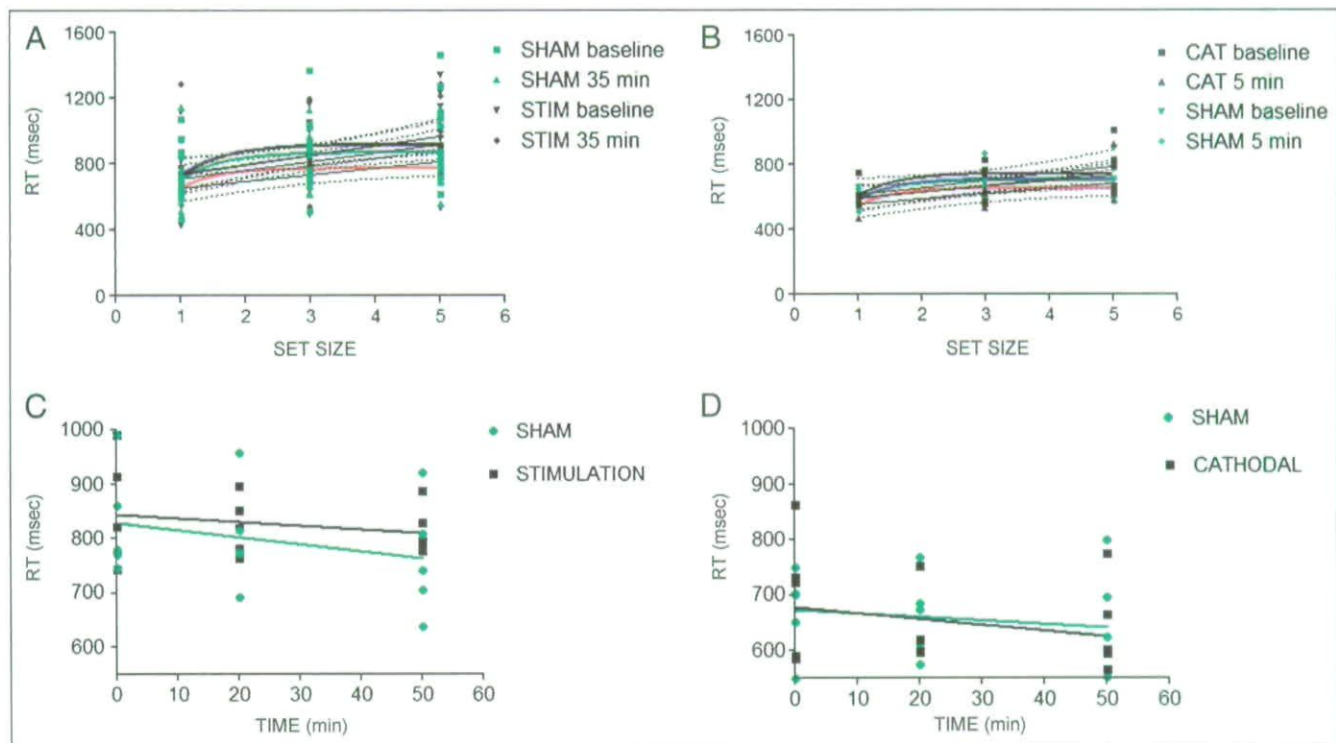


Figure 5. Plot of RTs as a function of set size for cerebellar tDCS (A) and for prefrontal stimulation (B). (C) Linear regression of RTs in experimental session time (from baseline to 35 min) during the stimulation of the cerebellum in the five subjects who underwent both cerebellar and prefrontal tDCS. The two lines represent the linear fit for the stimulation (black) and the sham (green) sessions. (D) Linear regression of RTs in experimental session time (from baseline to 35 min) during stimulation of the prefrontal cortex in the same subjects as in C. The two lines represent the linear fit for the cathodal tDCS (black) and the sham tDCS (green) sessions. Note that the slope is lower during cerebellar tDCS experiments than during sham stimulation.

RT changes (Stimulation site \times Stimulation type: $p = .0048$), thereby confirming that the effect of cathodal tDCS over the prefrontal cortex differed significantly from that of tDCS over the cerebellum.

In summary, cathodal tDCS over the prefrontal cortex significantly decreased RTs immediately after stimulation began (at 5 min), whereas anodal stimulation had no effect.

Learning Rate in the Sternberg Paradigm

The regression analysis used to evaluate the Sternberg VWM task learning function showed a linear trend in the time course of RTs probably corresponding to the linear approximation of a first-order exponential decay in the initial decoding phase, before reaching the plateau. Baseline RTs tended to decrease linearly across sessions, suggesting that task execution is shortened by learning mechanisms. The learning rate calculated over the entire test was -34.17 ± 10.2 . Learning rates were similar for present and absent answers (present: -51.93 ± 12.7 ; absent: -50.42 ± 10.0). Conversely, the learning rate was lower for the simplest VWM task than for the more complex tasks (Set size 1: -42.30 ± 9.7 ; Set size 3: 49.91 ± 12.5 ; Set size 5: -49.02 ± 15.3).

In the linear regression analysis, investigating RTs in experimental session time (from baseline to 35 min), during cerebellar tDCS, the comparison between the two slopes disclosed a significantly higher decreasing

rate for sham experiments than for stimulation experiments (-2.46 ± 2.49 vs. -0.75 ± 1.9 , $p = .02$). During tDCS over the prefrontal cortex, no significant difference was found between the decreasing rates for anodal, cathodal, and sham stimulation (anodal: -0.013 ± 1.11 ; cathodal: -1.034 ± 0.93 ; sham: -0.614 ± 1.18). The y-intercept of the slope calculated during tDCS over the prefrontal cortex was lower than that calculated during tDCS over the cerebellum. The difference probably depended on the learning-related linear decrease in baseline values across tDCS sessions (Figure 5).

The analysis of the RT/memory set size slope disclosed that the mean baseline slope for the overall sample was 49.26 ± 9.32 msec/digit comparable to the value obtained in previous studies (Archibald & Fisk, 2000). Whereas cerebellar tDCS increased the RT/memory size slope (tDCS vs. sham: 0.35 ± 1.48 vs. -0.22 ± 0.4 msec/digit), prefrontal tDCS decreased it (tDCS vs. sham: -0.21 ± 0.57 vs. 0.02 ± 0.34). After cerebellar tDCS, the RT/memory size slope improved slightly ($<1SD$) in only 3 of the 13 subjects, whereas after prefrontal tDCS, it improved in three of the five.

tDCS and Visual Evoked Potentials

ANOVA disclosed no significant effect of the four factors on P100 amplitudes (P100-N75, and P100-N145; Figure 6A and B). Nor did tDCS of the cerebellum affect the P100 latency (Figure 6C).

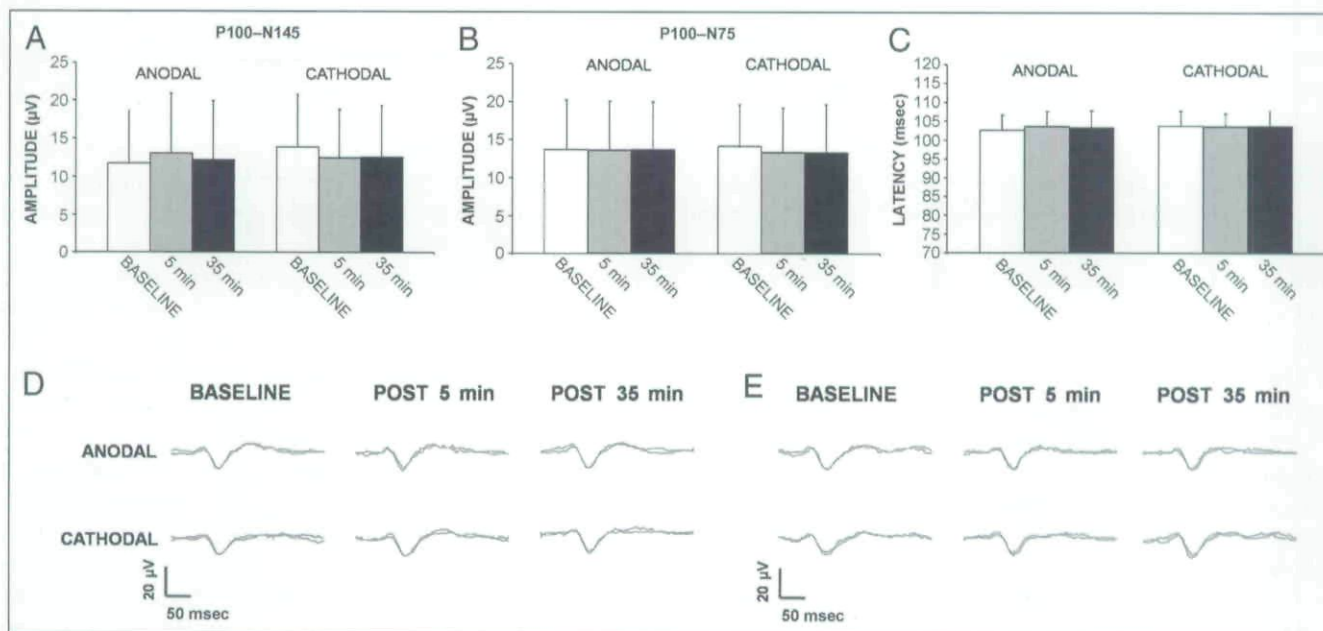


Figure 6. Cerebellar tDCS and VEPs. (A) The effects of anodal (left) and cathodal (right) tDCS on VEPs P100-N145 amplitude. Empty bars: baseline; gray bars: 5 min after the end of tDCS; black bars: 35 min after the end of tDCS. Bars are means; error bars are SD. (B) The effects of anodal (left) and cathodal (right) tDCS on VEPs P100-N75 amplitude. The rest of the legend as A. (C) The effects of anodal (left) and cathodal (right) tDCS on VEPs P100 latency; the rest of the legend as A. (D) and (E) Raw VEP recordings before and after tDCS in two representative subjects. In each panel: on the left, VEPs before tDCS; in the middle, VEPs 5 min after the end of tDCS; on the right, VEPs 35 min after the end of tDCS; top traces anodal tDCS, bottom traces cathodal tDCS. Note that cerebellar tDCS did not influence VEP amplitude and latency.

DISCUSSION

Our study provides previously unreported information that tDCS delivered over the cerebellum impairs the practice-dependent increase in VWM task proficiency, thus suggesting that the cerebellum is an important component of a circuitry involved in the practice-dependent proficiency increase in WM. Our experiments using VEPs and those delivering tDCS over the prefrontal cortex argue against an action of cerebellar tDCS on the visual system, therefore specifying that cerebellar tDCS induces structure-specific effects independent of visual system involvement.

Cerebellum and the Practice-dependent Increase in VWM Task Proficiency

Cerebellar tDCS impaired the practice-dependent decrease in RTs for the VWM task measured at 35 min after stimulation ended and also significantly changed task learning rates. Because the occipital cortex has been implicated in WM (Kandel, Kupfermann, & Iversen, 2000), tDCS over the cerebellum could, in theory, act by inducing changes also in the occipital cortex. Our experiments showing that cerebellar tDCS left VEPs unchanged argue against a role of the visual system in the occipital lobe as a possible source of impaired visual input to explain the effect on the Sternberg task. Although none of our subjects showed cerebellar motor impairment, some subtle, subclinical motor abnormality might have contributed in increasing the RT. Motor impairment is unlikely, however, because tDCS would then have increased the RT also at 5 min and not selectively at 35 min after stimulation. The lack of motor impairment is further supported by our observation that tDCS over the cerebellum left the slope of the linear fit RT/memory set size unchanged. Changes in the slope of this function reflect the speed for memory searching (Sternberg, 1969), whereas changes in the zero-intercept of the RT function reflect motor effects, such as effects on encoding the test stimulus and organizing and executing the response. Current knowledge is in line with the significant relationship observed by Archibald et al. (2004) between posterior fossa lesion volume and memory scanning speed. Conversely, in our experiments, prefrontal tDCS improved the speed of searching memory, thereby confirming a direct involvement of this cortex in WM mechanisms. Hence, the cerebellum controls the practice-dependent increase in VWM task proficiency.

Our finding that cathodal tDCS over the prefrontal cortex significantly decreased RTs agrees with current knowledge that the neuronal circuits for WM are at least, in part, located in the prefrontal cortex (attentional control system) (Kandel et al., 2000) and with previous studies generally showing that—despite some controversial results due to the specific methodologies issues—

tDCS over the frontal cortex alters the performance of a WM task (Boggio et al., 2006; Fregni et al., 2005; Marshall et al., 2005). The difference between our results and those reported by Boggio et al. (2006) and Fregni et al. (2005) could depend on several methodological issues. First, to avoid confounding biases arising from two electrodes with opposite polarities over the scalp, we used a noncephalic reference electrode (Priori et al., 2008; Cogiamanian et al., 2007). Conversely, Boggio et al. and Fregni et al. used a montage with the anode electrode placed over F3 and the cathode placed over the contralateral supraorbital area, thereby introducing an intracortical current path that could produce a different tDCS response. Second, for stimulation, we used an anodal or cathodal DC at 2 mA intensity delivered for 15 min, and tested subjects 5 min after stimulation ended, whereas Fregni et al. used a 1-mA DC delivered for 10 min, and Boggio et al. applied 2 mA for 20 min and both investigators tested subjects on-line in the final 5 min of stimulation. Because the total charge delivered differed in the three experiments the effects of tDCS evaluated after stimulation ended or during tDCS might also have differed. Third, we allowed a 7-day washout, whereas Fregni et al. used only 1 hr and Boggio et al. 48 hr. Residual long-lasting effects of tDCS could have influenced subjects' responses. Finally, Boggio et al. tested patients with Parkinson's disease who were probably far less accurate than healthy subjects. Conversely, our subjects could not improve accuracy because their mean accuracy at baseline was nearly 100%. Although prefrontal tDCS yielded shorter baseline RTs than cerebellar sessions, cathodal stimulation shortened them further, thereby indicating that task learning was not yet asymptotic. The distinctive finding in our study was that cathodal prefrontal tDCS shortened the RTs immediately at the 5 min assessment, elicited no additional after-effects at 35 min, and no change in the learning rate. Our observation that prefrontal tDCS induced an initial facilitation, but with no further increase in task proficiency, suggests a ceiling effect. The most important conclusion is that the changes in VWM induced by tDCS over the prefrontal cortex substantially differ from those elicited by cerebellar tDCS.

Our study using tDCS may also provide some insight into whether the cerebellum has a primary role in VWM, as Desmond et al. (2005) suggested by transiently interfering with cerebellar function in their study using TMS. A different issue is whether and how the cerebellum controls the practice-dependent increase in VWM task proficiency. The practice-dependent increase in the modified Sternberg paradigm performance we observed closely resembles that reported by Kirschen et al. (2005). Although they demonstrated an increased cerebro-cerebellar activation correlating with a practice-dependent increase, whether increased cerebellar activity is a primary cause or simply a hyperactivity corollary to frontal hyperactivation remained unclear. Our find-

ings imply that tDCS over the cerebellum elicits persistent after-effects on the cerebellar circuitry, not interfering with WM performance per se but ultimately and selectively impairing practice-related changes. We suggest that tDCS shifts the neurons in the cerebellar cortex away from their equilibrium for several minutes, thus interfering with their function, and ultimately, impairing practice-dependent cerebellar changes but not the WM per se.

Our experiments with cerebellar tDCS also provide insights into how practice affects cognitive functions. An unanswered question is why the RTs for the VWM paradigm were normal at 5 min but increased at 35 min after tDCS ended. A possible explanation is that practice exerts its effects on the cerebellar circuitry during the first trial, before tDCS has disrupted cerebellar function. In contrast, 35 min after cerebellar tDCS ends, RTs increase because tDCS interferes with the development of practice-related changes that would normally have taken place during the trial at 5 min after brain polarization began. Another possible explanation is that tDCS impairs cerebellar information processing tested during 5 min after tDCS began, affecting cerebellar control on task performance tested 35 min after tDCS ended. Cerebellar control action is organized in a feed-forward architecture, implying intermediate learning steps: Information needed to build up control schemas is processed by the cerebellum during process execution and is used to control subsequent tasks. During the first task tested 5 min after tDCS began, tDCS probably impairs cerebellar function, thereby changing the ability of the WM system to process correctly the information relative to the current task, but leaving the present control, derived from the preceding task execution, unaffected. The task tested at 35 min therefore relies on the sub-optimal information processed during the task tested at 5 min.

A further point is that tDCS elicited a greater effect on absent than on present answers. These changes became evident 35 min after tDCS ended. At first glance, absent answers might require more complex neural processing and, also, more complex cerebellar control than present answers: An absent response can only be made after all items in STM have been searched for and found not to match the probe item. A present trial could terminate as soon as the probe item is matched with the appropriate item in STM, thereby shortening the time required for answer processing. This hypothesis receives support from our results showing longer RTs for absent than for present answers. Our task should amplify this effect because we presented the memory set simultaneously as an array, thereby offering the opportunity to represent it as a visual image. Absent answers could require a complete recall of the memory set, whereas present answers could be given when the matching number is found in the memorized set. Insofar as cerebellar control is more complex for absent than for

present answers, cerebellar tDCS probably interferes more with absent than with present RTs.

tDCS and the Cerebellum

Our finding that tDCS delivered over the cerebellum elicits a long-lasting interference with cerebellar function receives support from several observations. At the scalp site, where we delivered tDCS, the cerebellum is more superficial than, for instance, the motor cortex below the fronto-parietal bone, because the occipital bone is thinner than the parietal bone (Axelsson, Kjaer, Heiberg, Bjornland, & Storhaug, 2005). Computational simulations based on the three-sphere model of the head (Miranda et al., 2006) show that approximately half of the current is shunted through the scalp and concentrated in the region surrounding the electrode edge (with squared electrodes). In our experimental setting, we minimized edge effects by using elliptical electrodes. The portion of the current not shunted through the scalp enters the skull + scalp below the stimulating electrodes (Miranda et al., 2006) and is directed to the lowest-impedance path toward the reference electrode, placed on the right arm (Holdefer, Sadleir, & Russell, 2006). To do so, the current flows tangentially to the cerebellar surface. The cerebellar cortex contains fibers running parallel to the cortical surface and, therefore, maximally affected by tangential currents. Also, the impedance of neural elements in the cerebellar cortex is lower than that of elements in the motor cortex. For instance, in the rat, input impedance of Purkinje cells is about one third of that of pyramidal cells in the motor cortex (Zhu, Scelfo, Tempia, Sacchetti, & Strata, 2006; Jacobson et al., 2005), leading to larger current flow through the cell membrane of Purkinje cells in the cerebellar cortex than through pyramidal cells in the motor cortex. A final observation arguing that tDCS can influence cerebellar function is that the tDCS-induced changes in cerebral cortical excitability resemble those induced by repetitive TMS (rTMS) (Fregni et al., 2006), a technique known to affect the cerebellum (Oliveri et al., 2007). For these reasons, we conclude that the cerebellum not only can be influenced by tDCS but is the only plausible structure to be affected in our experiments.

Our findings imply that cerebellar tDCS influences VWM task by interfering the increase in cerebellar activation. This hypothesis fits in with the observation that tDCS over the motor cortex can suppress the normal activation pattern in motor cortical areas (Baudewig, Nitsche, Paulus, & Frahm, 2001), specifically affecting responses involving "cortico-cortical processing." These mechanisms can be relevant also for tDCS over the cerebellum. A final open question is how tDCS affects cerebellar function. It could do so in at least two ways. First, tDCS could interfere with long-term depression (LTD) (Paulus, 2004). LTD of Purkinje cells can play a role not only in motor function but also in cognitive

tasks (Vigot, 2003). Hence, tDCS of both polarities could alter the fine tuning of the membrane potential needed for LTD. A further possibility is that tDCS could directly alter the membrane properties of the neuronal elements in the cerebellar cortex. In rats, changes in membrane properties and of ionic conductances, in turn, lead to changes in Purkinje cell intrinsic pacemaking and, ultimately, interfere with their synaptic information (Womack & Khodakhah, 2002), perturbing the signal processing in the cerebellar cortex. Abnormalities of intrinsic pacemaking mechanisms of the Purkinje cells result in cerebellar deficits, and normal cerebellar function can be restored when pacemaking is normalized (Walter, Alvina, Womack, Chevez, & Khodakhah, 2006). By analogy, we therefore argue that the subtle impairment of intrinsic Purkinje cell pacemaking induced by tDCS interferes with their processing of cognitive information. Finally, the cerebellum could control the neural circuits responsible for VWM through its efferent projections to the frontal lobe. Yet, cerebellar stimulation in humans elicits consistent electroencephalogram changes in the contralateral brain hemisphere (Snider & Wetzel, 1965).

Although tDCS over the motor cortex induces polarity-specific opposite changes in excitability (Nitsche & Paulus, 2000), in our experiments, we found no difference between the anodal and cathodal tDCS over the cerebellum. This observation agrees with the lack of polarity-specific tDCS-induced changes in cognitive experiments (Marshall et al., 2005). A possible explanation for the lack of polarity specificity of cerebellar tDCS comes from general physiological mechanisms that have been known for years (Lorente De Nò, 1947). The loss of function of any excitable tissue can be obtained both with depolarization and hyperpolarization. For instance, classic neurophysiological experiments demonstrated that axonal conduction can be completely blocked, even for several hours, both by depolarization ("depolarizing" block) and by hyperpolarization ("hyperpolarizing" or "anodal" block), both leading to the same decreased excitability and, ultimately, to a loss of function (Lorente De Nò, 1947). This lack of polarity specificity could well apply also to the cerebellum, a brain structure that is theoretically more susceptible to direct current (see above) and has a hierarchically superior role in controlling cortical processing.

In line with Marsden, Merton, Morton, Hallet, and Adam (1977), we failed to observe changes in cerebellar motor function during or after cerebellar tDCS. This implies that tDCS leaves cerebellar motor functions, possibly located in the deepest part of this structure, and thus, less accessible to the effects of polarizing currents, unchanged. This conclusion agrees also with the observation that, in general, disorders selectively involving the cerebellar cortex manifest with mild and transient clinical signs.

In conclusion, our results show that tDCS can modulate the function of the cerebellum and that this is an

important structure for the development of neuronal changes induced by practice leading to increased proficiency of non-motor tasks.

Acknowledgments

We thank Professor Saul Sternberg for his helpful comments on a preliminary version of the manuscript. The work has been supported by Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, and by the University of Milan, Italy.

Reprint requests should be sent to Alberto Priori, Dipartimento di Scienze Neurologiche, Università degli Studi di Milano, Ospedale Maggiore Policlinico, Padiglione Ponti, V. F. Sforza 35, Milan, 20122 Italy, or via e-mail: alberto.priori@unimi.it.

REFERENCES

- Accornero, N., Li Voti, P., La Riccia, M., & Gregori, B. (2007). Visual evoked potentials modulation during direct current cortical polarization. *Experimental Brain Research*, 178, 261–266.
- Allen, G., Muller, R. A., & Courchesne, E. (2004). Cerebellar function in autism: Functional magnetic resonance image activation during a simple motor task. *Biological Psychiatry*, 56, 269–278.
- Archibald, C. J., & Fisk, J. D. (2000). Information processing efficiency in patients with multiple sclerosis. *Journal of Clinical and Experimental Neuropsychology*, 22, 686–701.
- Archibald, C. J., Wei, X., Scott, J. N., Wallace, C. J., Zhang, Y., Metz, L. M., et al. (2004). Posterior fossa lesion volume and slowed information processing in multiple sclerosis. *Brain*, 127, 1526–1534.
- Ardolino, G., Bossi, B., Barbieri, S., & Priori, A. (2005). Non-synaptic mechanisms underlie the after-effects of cathodal transcutaneous direct current stimulation of the human brain. *Journal of Physiology*, 568, 653–663.
- Axelsson, S., Kjaer, I., Heiberg, A., Bjornland, T., & Storhaug, K. (2005). Neurocranial morphology and growth in Williams syndrome. *European Journal of Orthodontics*, 27, 32–47.
- Baddeley, A. (1992). Working memory. *Science*, 255, 556–559.
- Baudewig, J., Nitsche, M. A., Paulus, W., & Frahm, J. (2001). Regional modulation of BOLD MRI responses to human sensorimotor activation by transcranial direct current stimulation. *Magnetic Resonance in Medicine*, 45, 196–201.
- Boggio, P. S., Ferrucci, R., Rigonatti, S. P., Cobre, P., Nitsche, M., Pascual-Leone, A., et al. (2006). Effects of transcranial direct current stimulation on working memory in patients with Parkinson's disease. *Journal of the Neurological Sciences*, 249, 31–38.
- Cedzich, C., Pechstein, U., Schramm, J., & Schafer, S. (1998). Electrophysiological considerations regarding electrical stimulation of motor cortex and brain stem in humans. *Neurosurgery*, 42, 527–532.
- Chen, S. H., & Desmond, J. E. (2005). Cerebrocerebellar networks during articulatory rehearsal and verbal working memory tasks. *Neuroimage*, 24, 332–338.
- Cogiamanian, F., Marceglia, S., Ardolino, G., Barbieri, S., & Priori, A. (2007). Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas. *European Journal of Neuroscience*, 26, 242–249.

- Desmond, J. E., Chen, S. H., DeRosa, E., Pryor, M. R., Pfefferbaum, A., & Sullivan, E. V. (2003). Increased frontocerebellar activation in alcoholics during verbal working memory: An fMRI study. *Neuroimage*, 19, 1510–1520.
- Desmond, J. E., Chen, S. H., & Shieh, P. B. (2005). Cerebellar transcranial magnetic stimulation impairs verbal working memory. *Annals of Neurology*, 58, 553–560.
- Fiez, J. A. (1996). Cerebellar contributions to cognition. *Neuron*, 16, 13–15.
- Fiez, J. A., Petersen, S. E., Cheney, M. K., & Raichle, M. E. (1992). Impaired non-motor learning and error detection associated with cerebellar damage. A single case study. *Brain*, 115, 155–178.
- Fregni, F., Boggio, P. S., Nitsche, M., Bormpohl, F., Antal, A., Feredoes, E., et al. (2005). Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. *Experimental Brain Research*, 166, 23–30.
- Fregni, F., Marcondes, R., Boggio, P. S., Marcolin, M. A., Rigonatti, S. P., Sanchez, T. G., et al. (2006). Transient tinnitus suppression induced by repetitive transcranial magnetic stimulation and transcranial direct current stimulation. *European Journal of Neurology*, 13, 996–1001.
- Gordon, N. (2007). The cerebellum and cognition. *European Journal of Paediatric Neurology*, 11, 232–234.
- Holdefer, R. N., Sadleir, R., & Russell, M. J. (2006). Predicted current densities in the brain during transcranial electrical stimulation. *Clinical Neurophysiology*, 117, 1388–1397.
- Jacobson, G. A., Diba, K., Yaron-Jakubovitch, A., Oz, Y., Koch, C., Segev, I., et al. (2005). Subthreshold voltage noise of rat neocortical pyramidal neurones. *Journal of Physiology*, 564, 145–160.
- Kandel, E. R., Kupfermann, I., & Iversen, S. (2000). Learning and memory. In E. R. Kandel, J. H. Schwartz, & T. M. Jessel (Eds.), *Principles of neural sciences* (4th ed., pp. 1227–1246). New York: McGraw-Hill.
- Kirschen, M. P., Chen, S. H., Schraedley-Desmond, P., & Desmond, J. E. (2005). Load- and practice-dependent increases in cerebello-cerebellar activation in verbal working memory: An fMRI study. *Neuroimage*, 24, 462–472.
- Liebetanz, D., Nitsche, M. A., Tergau, F., & Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain*, 125, 2238–2247.
- Lorente De Nò, R. (1947). *A study of nerve physiology*. New York: Rockefeller Institute for Medical Research.
- Marsden, C. D., Merton, P. A., Morton, H. B., Hallett, M., Adam, J., & Rushton, D. N. (1977). Disorders of movement in cerebellar disease in man. In F. C. Rose (Ed.), *Physiological aspects of clinical neurology* (pp. 179–199). Oxford: Blackwell.
- Marshall, L., Molle, M., Siebner, H. R., & Born, J. (2005). Bifrontal transcranial direct current stimulation slows reaction time in a working memory task. *BMC Neuroscience*, 6, 23.
- Middleton, F. A., & Strick, P. L. (1994). Anatomical evidence for cerebellar and basal ganglia involvement in higher cognitive function. *Science*, 266, 458–461.
- Miranda, P. C., Lomarev, M., & Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clinical Neurophysiology*, 117, 1623–1629.
- Nathan, S. S., Sinha, S. R., Gordon, B., Lesser, R. P., & Thakor, N. V. (1993). Determination of current density distributions generated by electrical stimulation of the human cerebral cortex. *Electroencephalography and Clinical Neurophysiology*, 86, 183–192.
- Nitsche, M. A., Liebetanz, D., Lang, N., Antal, A., Tergau, F., & Paulus, W. (2003). Safety criteria for transcranial direct current stimulation (tDCS) in humans. *Clinical Neurophysiology*, 114, 2220–2222; author reply 2222–2223.
- Nitsche, M. A., & Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *Journal of Physiology*, 527, 633–639.
- Oliveri, M., Torriero, S., Koch, G., Salerno, S., Petrosini, L., & Caltagirone, C. (2007). The role of transcranial magnetic stimulation in the study of cerebellar cognitive function. *Cerebellum*, 6, 95–101.
- Paulus, W. (2004). Outlasting excitability shifts induced by direct current stimulation of the human brain. *Supplements to Clinical Neurophysiology*, 57, 708–714.
- Priori, A. (2003). Brain polarization in humans: A reappraisal of an old tool for prolonged non-invasive modulation of brain excitability. *Clinical Neurophysiology*, 114, 589–595.
- Priori, A., Berardelli, A., Rona, S., Accornero, N., & Manfredi, M. (1998). Polarization of the human motor cortex through the scalp. *NeuroReport*, 9, 2257–2260.
- Priori, A., Bossi, B., Ardolino, G., Bertolasi, L., Carpo, M., Nobile-Orazio, E., et al. (2005). Pathophysiological heterogeneity of conduction blocks in multifocal motor neuropathy. *Brain*, 128, 1642–1648.
- Priori, A., Mameli, F., Cogiamanian, F., Marceglia, S., Tiriticco, M., Mrakic-Sposta, S., et al. (2008). Lie-specific involvement of dorsolateral prefrontal cortex in deception. *Cerebral Cortex*, 18, 451–455.
- Snider, R. S., & Wetzell, N. (1965). Electroencephalographic changes induced by stimulation of the cerebellum of man. *Electroencephalography and Clinical Neurophysiology*, 18, 176–183.
- Sternberg, S. (1966). High-speed scanning in human memory. *Science*, 153, 652–654.
- Sternberg, S. (1969). Memory-scanning: Mental processes revealed by reaction-time experiments. *American Scientist*, 57, 421–457.
- Torriero, S., Oliveri, M., Koch, G., Lo Gerfo, E., Salerno, S., Petrosini, L., et al. (2007). Cortical networks of procedural learning: Evidence from cerebellar damage. *Neuropsychologia*, 45, 1208–1214.
- Van Mier, H. I., & Petersen, S. E. (2002). Role of the cerebellum in motor cognition. *Annals of the New York Academy of Sciences*, 978, 334–353.
- Vigot, R. (2003). Cerebellar long-term depression: A mechanism for learning and memory. *Medical Science (Paris)*, 19, 437–441.
- Walter, J. T., Alvina, K., Womack, M. D., Chevez, C., & Khodakhah, K. (2006). Decreases in the precision of Purkinje cell pacemaking cause cerebellar dysfunction and ataxia. *Nature Neuroscience*, 9, 389–397.
- Wassermann, E. M., & Grafman, J. (2005). Recharging cognition with DC brain polarization. *Trends in Cognitive Sciences*, 9, 503–505.
- Womack, M., & Khodakhah, K. (2002). Active contribution of dendrites to the tonic and trimodal patterns of activity in cerebellar Purkinje neurons. *Journal of Neuroscience*, 22, 10603–10612.
- Zhu, L., Scelfo, B., Tempia, F., Sacchetti, B., & Strata, P. (2006). Membrane excitability and fear conditioning in cerebellar Purkinje cell. *Neuroscience*, 140, 801–810.

Copyright of Journal of Cognitive Neuroscience is the property of MIT Press and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.