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Anodal Transcranial Direct Current Stimulation Enhances Procedural Consolidation

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Tecchio F, Zappasodi F, Assenza G, Tombini M, Vollaro S, Barbati G, Rossini PM. Anodal transcranial direct current stimulation enhances procedural consolidation. *J Neurophysiol* 104: 1134–1140, 2010. First published June 10, 2010; doi:10.1152/jn.00661.2009. The primary motor cortex (M1) area recruitment enlarges while learning a finger tapping sequence. Also M1 excitability increases during procedural consolidation. Our aim was to investigate whether increasing M1 excitability by anodal transcranial DC stimulation (AtDCS) when procedural consolidation occurs was able to induce an early consolidation improvement. Forty-seven right-handed healthy participants were trained in a nine-element serial finger tapping task (SFTT) executed with the left hand. Random series blocks were interspersed with training series blocks. Anodal or sham tDCS was administered over the right M1 after the end of the training session. After stimulation, the motor skills of both trained and a new untrained sequential series blocks were tested again. For each block, performance was estimated as the median execution time of correct series. Early consolidation of the trained series, assessed by the performance difference between the first block after and the last block before stimulation normalized by the random, was enhanced by anodal and not by sham tDCS. Stimulation did not affect random series execution. No stimulation effect was found on the on-line learning of the trained and new untrained series. Our results suggest that AtDCS applied on M1 soon after training improves early consolidation of procedural learning. Our data highlight the importance of neuromodulation procedures for understanding learning processes and support their use in the motor rehabilitation setting, focusing on the timing of the application.

INTRODUCTION

As opposed to declarative memory, i.e., the memory of events or notions, motor or procedural learning refers to the process by which simple or complex movements are acquired through practice. To study motor learning in the laboratory, two different experimental paradigms are usually addressed: movements within a repeated fixed behavioral pattern (motor sequence learning; Karni et al. 1995, 1998) or movements to compensate for environmental changes (motor adaptation; Broussard and Kassardjian 2004; Shadmehr and Holcomb 1997). In particular, serial finger movements are a well-known task used to evaluate procedural learning, with the possibility of investigating explicit/implicit procedural learning counterparts, which are the acquisition of certain skills with/without the awareness of the rule behind the task. Motor sequence learning is usually measured by an increase of speed (as evidenced by reduction of reaction or execution time) or accuracy (as usually expressed by reduction of number of errors). Psychophysical studies have demonstrated that the incremental acquisition of motor skills follows distinct stages: first, an initial stage of learning in which considerable within-session improvement in performance can be seen on a time-scale of minutes for a task never trained before (fast on-line learning); later, further incremental gains can be observed after successive sessions of practice (slow on-line learning; Karni et al. 1998; Nudo et al. 1996; Reis et al. 2009). In the intermediate period between consecutive sessions different phenomena (off-line effects; Robertson et al. 2004a; Stickgold et al. 2001) can occur, with a worsening (Reis et al. 2009), maintenance, or an amelioration (Brashers-Krug et al. 1996; Hotermans et al. 2006; Karni et al. 1998) of the performance reached through practice. The phenomenon of continuing improvement in a skill even without exercise is called off-line learning or consolidation (James 1890; Krakauer and Shadmehr 2006; Robertson et al. 2004b). Besides well-known learning modulations that take place in several hours and days (Krakauer et al. 2005), with a complex dependence on wakefulness and sleep stages (Luft and Buitrago 2005), it has been recently demonstrated that consolidation also occurs in the first minutes after the end of a motor training session (early consolidation; Muellbacher et al. 2002).

During the past decade, neuroimaging and behavioral studies contributed to the understanding of the neural substrate subtending procedural learning, showing a prominent role of cortical primary motor area (M1; Karni et al. 1995, 1998; Muellbacher 2001; Richardson et al. 2006; Robertson et al. 2005; Walker 2005). M1 was demonstrated to participate in both fast on-line learning (Karni 1995; Ungerleider et al. 2002) and early consolidation stages (Muellbacher et al. 2002). In fact, Karni and collegues (1995) demonstrated a growing M1 recruitment during the training phase of a fixed finger-tapping sequence. In parallel to an enlargement of recruited M1 area (Karni et al. 1995; Pascual Leone et al. 1995) neurophysiological studies demonstrated that M1 neuronal pools involved in the learned task increased excitability during motor learning (Liepert et al. 1998; Muellbacher et al. 2001; Perez et al. 2007).

Transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS) are widely used techniques able to modulate cortical excitability. In particular, it has been

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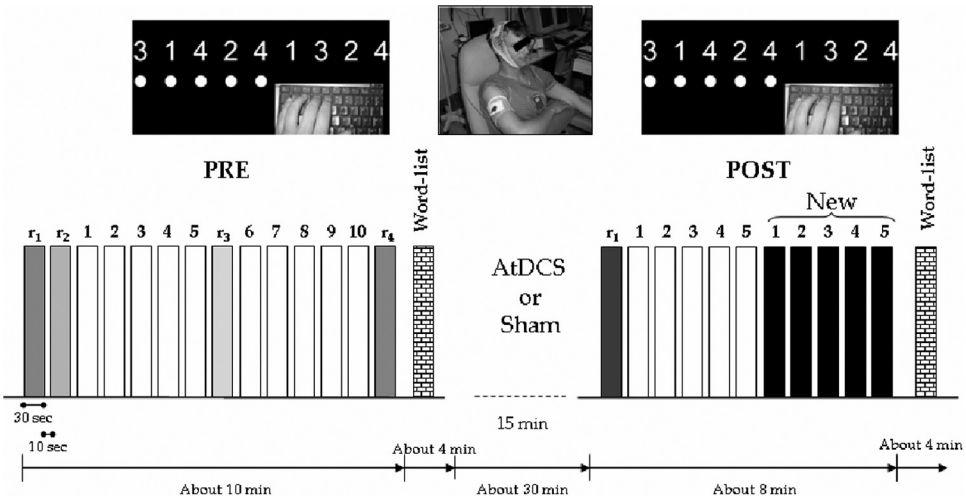

FIG. 1. Experimental paradigm. *Top*: modified serial finger tapping task (SFTT): the series appeared on the PC screen to be performed with the left hand; placement of transcranial direct current stimulation (tDCS) electrodes. *Bottom*: diagram showing the presentation order of the trained sequential (PRE/POST; white) and untrained sequential ($POST_{NEW}$; black) and random series (r; gray) blocks. Indicative timing is shown.

shown that tDCS increases cortical excitability of the area beneath the anodic electrode (Nitsche and Paulus 2000, 2001). Inhibition of M1 cortical excitability via slow repetitive TMS immediately after motor practice disrupted early consolidation of the motor improvements (Muellbacher et al. 2002). The aim of our study was to assess whether increasing M1 excitability by anodal tDCS (AtDCS) immediately after the training session was able to induce an early consolidation improvement. A serial finger tapping task (SFTT) was used. The AtDCS influence on on-line learning in the poststimulation session was also addressed.

METHODS

Subject and tDCS procedure

Forty-seven healthy right-handed participants (mean age 29 ± 5 yr, 22 females, Edinburgh manuality median 80, [39–100] 5%–95% percentile) were enrolled in the study. All participants were not taking any CNS-affecting drug. Subjects' recruitment was performed to obtain two groups matched by gender, age, and playing of musical instruments. Moreover, prior to directing the subject to real anodal (AtDCS) or sham (Sham) tDCS, manual dexterity estimated by performance level in the training period was also matched. Each subject participated in only one session (AtDCS or Sham). DC (1 mA) was delivered for 15 min by a battery-driven stimulator (Phoresor II, model PM700; Motion Control, Salt Lake City, UT) through saline-soaked sponge electrodes (surface area: 7 × 5 cm^2). The active electrode was positioned over the right primary motor cortex (centered over C4 scalp position of the International 10/20 System, the 7 cm long side along the central sulcus, the 5 cm long side across it, i.e., overlying the precentral postcentral regions) and the reference above the ipsilateral arm (Cogiamanian et al. 2007; Fig. 1). The current flow was checked by a voltmeter. Since the stimulation can cause a short-lasting tingling during the transient period of current turn-on and turn-off, Sham stimulation was realized by delivering current for 10 s at both the start and the end of the 15 min.

Experimental paradigm

SETTINGS AND TASK. Participants were comfortably seated in front of a computer screen at eye level, with the left elbow and wrist positioned on a horizontal plane (Fig. 1). The left (nondominant) hand was located over the four buttons numbered 1–4 of a standard PC keyboard. A modified version of the SFTT (Walker et al. 2002) was used to test the acquisition of finger-movement series. The presentation of each stimulus was controlled by computer software. Each subject was instructed to repeat, as many times as possible in 30 s and as accurately as possible, a nine-element series (numbers from 1 to 4) displayed on the screen, by pushing the corresponding button with the corresponding finger (little finger for button 1, ring finger for 2, middle finger for 3, index finger for 4; Fig. 1). Subjects were instructed not to correct occasional errors but to continue with the task without pause. Typing advancing after each button press was indicated by a circle mark appearing below the corresponding number independently of the correctness of the typing, so that no accuracy feedback was provided to the subjects (Fig. 1). Along the 30 s block, the sequence was permanently displayed on the screen; circle marks disappeared after each of the nine button presses. An interval of 10 s followed each 30 s block.

FINGER TAPPING SERIES DESCRIPTION. The first block of random series was provided to make the subject confident with the keyboard and the task (R1, Fig. 1). A second random series block (R2) was used to assess the initial motor performance of subjects (Perez et al. 2007). In the training prestimulation period, two sets of five blocks with the same sequential series [3–1–4–2–4–1–3–2–4] were presented (PRE_{1-5} and PRE_{6-10}; Figs. 1 and 2), intermingled by random series blocks (R3, R4). R3 and R4 were series different from the sequential one [3–1–4–2–4–1–3–2–4] and from each other, remaining displayed on the screen for 30 s. Participants were not told about the order in which the different blocks would be presented (random or sequential). After stimulation, a new random series ($POST_{r1}$), five blocks of the same trained sequential series ($POST_{1-5}$) and five blocks of a new untrained sequential series [1–3–4–2–3–1–2–4–3] ($POST_{NEW1-5}$) were consecutively presented.

DECLARATIVE MEMORY CONTROL TASK. Just before stimulation and at the end of the SFTT after stimulation (Fig. 1), we used a

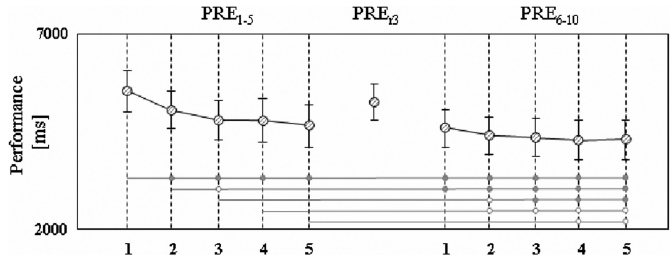

FIG. 2. Training mean performance (±SE) across all subjects for each sequence block preceding stimulation. Circle on horizontal lines indicate statistical difference between the performance of that block and the one where the line starts (Bonferroni corrected, $P < 0.01$ full circle, $P < 0.05$ empty circle). Performance improved to a stable level, reached at the PRE_6 sequence.

paired-associated word list learning task (PAWT) to evaluate declarative learning. Fifteen pairs of semantically related words were consecutively presented (modified version from Plihal and Born 1997). Each word pair, consisting of a stimulus word and an associated (response) word, was matched in length, emotionality, meaningfulness, and concreteness. Word pairs were individually and randomly presented on a computer screen (1 s for each word pair, 500-ms interstimulus interval). Subjects were requested to read each word silently and memorize the pairs. After presentation of the complete 15-pair list, cued recall was tested. In this phase of the declarative memory task, the stimulus word (the first word for each pair) appeared on the screen in a different random sequence. For each stimulus word, the subject was required to name the second word of the pair, with unlimited response time. Two different sets of word pairs were used before and after stimulation, equaled for frequency and difficulty. The whole task lasted about 4 min.

The informed consent was collected by each subject in participating to the protocol previously approved by the Fatebenefratelli Hospital Ethical committee.

Data analysis

Subjects were excluded from further analysis if they had produced >70% of wrong sequences during the prestimulation blocks. Trials with wrong sequences were excluded from further analysis. The median execution time of correct series was estimated as the performance index of each block. The number of incorrect sequences (accuracy) per block was also evaluated.

GENERAL LINEAR MODEL $(GLM)_{TRAINING}$. To quantify training effects, performances of the prestimulation session were submitted to a repeated-measures ANOVA model including *Training* ($PRE_{1–10}$) as the 10-level within-subjects factor.

$GLM_{CONSOLIDATION}$. To investigate possible effects of AtDCS on consolidation, the motor performances during the PRE_{10} and $POST_1$ sequences were submitted to a repeated-measures ANOVA model considering *Stimulation Type* (Sham, AtDCS) as the between-subjects factor and *Stimulation* (PRE-stimulation, POST-stimulation) as the within-subjects factor. To confirm that consolidation effects appeared selectively in trained sequences, we included the within-subjects factor *Series Type* (Random, Trained sequential), looking for the interaction effect *Series Type* × *Stimulation*. If this interaction factor was significant, we controlled that no consolidation appeared on random series by applying the above model only on random series (obviously omitting the *Series Type* factor; GLM_{Random}). After these checks, execution times of trained sequential series were normalized with respect to random ones. The new normalized variables, i.e.

$$POST_1^{norm} = \frac{POST_1 - POST_{r1}}{\frac{POST_1 + POST_{r1}}{2}}$$

and similarly

$$PRE_{10}^{norm} = \frac{PRE_{10} - PRE_{r4}}{\frac{PRE_{10} + PRE_{r4}}{2}}$$

were submitted to the corresponding $GLM_{NormalizedSequential}$ model, i.e., $GLM_{Consolidation}$ without *Series Type* factor.

$GLM_{ONLINELEARNING}$. To study the effects of Sham or AtDCS on on-line learning, the GLM was applied to the performances of the trained ($POST_{1–5}$) and the new untrained ($NEW_{1–5}$) poststimulation sequential series. Thus the *Stimulation Type* was the between-subjects factor and the two-level *Sequential Series Type* (Trained, New untrained) and five-level *On-line learning* were the within-subjects factors.

RESULTS

Among the 47 recruited subjects, 44 were available for the analysis, 22 belonging to the Sham, and 22 to the AtDCS groups. The remaining 3 subjects were excluded since, despite the simplicity of the required task, they made too many errors during the prestimulation sequences (>70% of wrong series). Gender and age were still homogeneously distributed between the two groups (10 females in Sham and 12 in AtDCS group; mean ± SD age: 29.0 ± 5.5 and 29.0 ± 5.3 yr, *F*-test, $P = 0.993$). As well, the Sham and AtDCS groups were balanced for the use of musical instruments (10 players in Sham and 8 in AtDCS group, chi-square Fisher's exact test, $P = 0.760$) and for the performance level in the training period (4.7 ± 0.3 and 4.3 ± 0.3 s, respectively, ANOVA on $PRE_{6–10}$, $P = 0.378$). To remove intrasubject variability of the performance at baseline, all data were normalized for the performance of the PRE_{r2} sequence.

Experiment timing

The entire prestimulation section lasted about 15 min, with the last 4 min devoted to the declarative memory task (Fig. 1). The time interval between the last sequence prestimulation (PRE_{r4}) and the first after stimulation ($POST_{r1}$) lasted 34 ± 6 min, with no difference between the Sham and AtDCS groups ($P > 0.200$). The poststimulation section lasted about 15 min.

Behavioral data description

At the end of the pre- and post-stimulation blocks no one noticed a repeating pattern.

Series errors. Unless the three excluded subjects, all others performed trained sequences with negligible errors. Consistently with low error levels, no effect was found on the percentage of incorrect series, either between pre- and poststimulation values, or between sequential or random series, or between AtDCS and Sham groups (consistently $P > 0.200$). On average, the nine-element sequential series were repeated five to six times for trained sequential, four to six times for new sequential, and four times for random series (Table 1).

Training. $GLM_{training}$ showed that, as expected, a significant reduction of performance execution time (Fig. 2) was found in the training sequences. In fact, a significant *Training* effect [Greenhouse–Geisser corrected, $F(4.0,156.7) = 27.710$, $P < 0.001$] was found, corresponding to an execution time decrease until the second block of the second set of trained sequences (PRE_7). After that no significant differences were present (Figs. 2 and 3). As a check, we repeated the ANOVA design with the *Training* within-subject factor at only four levels, on the performances from the seventh to the tenth PRE sequences: the *Training* effect was no longer significant [$F(3,117) = 0.787$, $P = 0.503$].

Consolidation. $GLM_{consolidation}$ model indicated, as expected, a different consolidation of random and trained sequential series [*Series Type* × *Stimulation*, $F(1,39) = 4.911$, $P = 0.033$]. No effect of consolidation was found for the random sequences (GLM_{Random} consistently $P > 0.300$).

TABLE 1. *Number of repetitions of the nine-element series*

	PRE			POST				
	Trained Sequential		Random	Random	Trained Sequential		New Sequential	
	PRE_6	PRE_{10}	PRE_{r4}	$POST_{r2}$	$POST_1$	$POST_5$	$POST_{NEW1}$	$POST_{NEW5}$
Sham	6 [2–12]	5 [1–14]	5 [3–9]	4 [1–10]	6 [1–14]	7 [2–14]	4 [1–10]	5 [1–12]
AtDCS	5 [1–11]	5 [1–11]	5 [1–8]	4 [1–9]	6 [1–13]	7 [1–15]	4 [1–11]	6 [1–13]

Median (minimum–maximum) of the number of repetitions of the correct nine-element series in one 30 s block for the two groups.

We also noted that different variances of performances were present in the AtDCS and Sham groups [Box's test of equality of covariance matrices in $GLM_{Consolidation}$, $F = 2.028$, $P = 0.027$], driven by the poststimulation series.

AtDCS and Sham induced different effects on consolidation [$GLM_{NormalizedSequential}$ *Stimulation Type* × *Stimulation* $F(1,39) = 4.417$, $P = 0.042$]. This was due to an amelioration of performances after AtDCS stimulation [paired-sample t-test, $t(18) = 2.839$, $P = 0.011$], which did not occur after Sham ($P = 0.665$) (Fig. 4).

To have a comparative estimate, we compared the dimension of the effect of consolidation enhancement due to AtDCS with that of the effect of changes due to training. The percentage change of execution time due to initial training was estimated as

$$\text{Training Effect} = \frac{PRE_1 - PRE_6}{PRE_1}$$

which was PRE_6, the end of training, since it was the last repetition for which people ameliorated the performance, i.e., they reduced the execution time. The training effect was 16%. The consolidation effect was correspondingly estimated as

$$\text{Consolidation Effect} = \frac{POST_1 - PRE_{10}}{PRE_{10}}$$

resulting in 5% for Sham (not reaching statistical significance) and 11% for AtDCS.

On-line learning

No effect was evidenced by the $GLM_{OnlineLearning}$ (Fig. 3).

Declarative learning

No effect was found on the percentage correct words-pair (consistently $P > 0.200$).

DISCUSSION

The present investigation proved that contralateral M1 anodal tDCS (AtDCS) applied soon after motor training facilitates early consolidation. In particular, we observed that these boosting effects were absent for random series, proving to be specific for consolidation, not influencing the motor act per se. Our results suggest that increasing the cortical excitability by an external intervention when early consolidation occurs strengthens synaptic transmission in those neural networks previously selected by training, enhancing the off-line retention.

AtDCS effects on consolidation without sleep

In Reis and colleagues (2009), M1 AtDCS applied during task execution induced selectively off-line positive effects of a movement series repeated on different days. Their experimental paradigm left the open question of whether tDCS could induce consolidation in the absence of sleep, since they did not repeat separate experimental sessions within the same day. Our data definitely provide an answer to this question, proving that similar M1 AtDCS can improve off-line consolidation without sleep. This result is of particular interest and we can hypothesize that despite the fact that AtDCS provides diffuse excitability modulation, the boosting sequence-specific effect could be observed if the intervention is applied when training cortical networks are already selected, thus strengthening by the tDCS stimulation cortical connections specifically involved in the task. In other terms, if the nonsynaptic-specific tDCS neuromodulation is provided when the specific synapses are already selectively strengthened by training, then neuromodulation will result in network-specific modulations. The dimension of the effect of consolidation enhancement due to AtDCS was not negligible with respect to training effect (11% vs. 16%). The huge variability of task execution capability across subjects induced effects that reached statistical significance in this

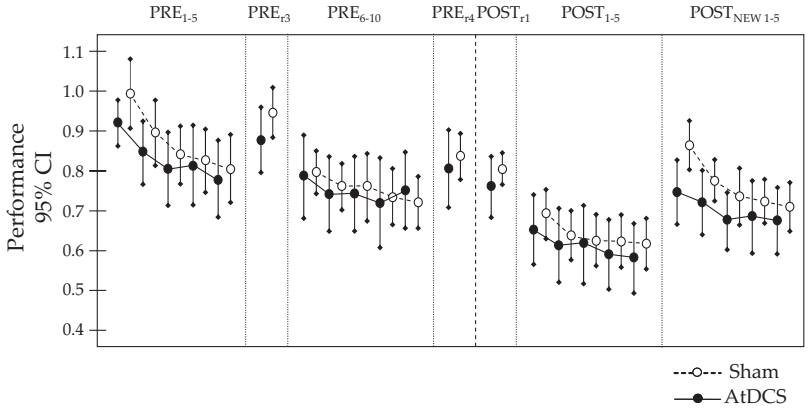

FIG. 3. Whole experiment performances. Execution times, normalized by PRE_{r2} values, along the whole experiment, differentiated as average (95% confidence interval [CI]) across subjects undergoing Sham (white circle) and anodal transcranial DC stimulation (AtDCS, black circle) stimulations.

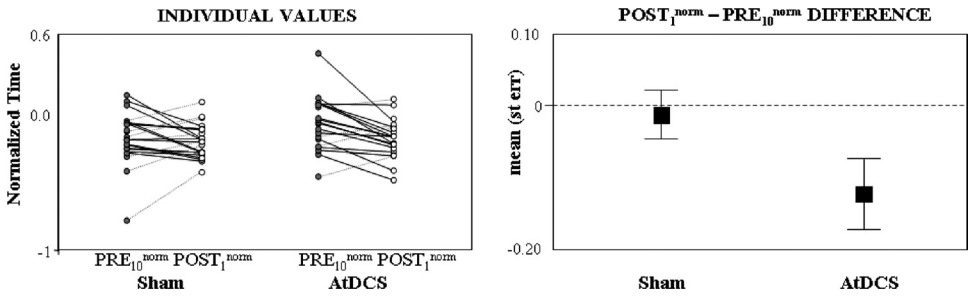

FIG. 4. Consolidation. *Left*: single subject performances in the last trained sequence block before (normalized PRE_{10}, gray circle) and the first after (normalized $POST_1$, white circle) stimulation. PRE and POST values in single subject were connected (full line for improved performance, dotted line for worsened). *Right*: mean and 2SEs of the normalized $POST_{10}^{norm} - PRE_{10}^{norm}$ difference for Sham and AtDCS stimulation.

group of 47 people, whereas we had in a first step of the study investigated 30 subjects and the behavior was present but did not reach statistical significance. Although confirmation of the behavior in the two groups makes us quite confident of the soundness of the result, the necessity of a large sample underlines that these procedural memory enhancements are miniscule in healthy people. Meanwhile, we were somehow impressed that the consolidation changes were similar in size to training changes. Such boosting effects present in healthy subjects pose good premises for treatments in stroke patients, who undergo huge relearning cerebral reorganization processes.

We are well aware that crucial control experiments would strengthen our confidence that the AtDCS effects are on consolidation and not on motor ability per se. Mainly two control conditions would be of interest: *1*) to modify the timing of the AtDCS stimulation, delaying the administration when consolidation processes are over; and *2*) to give the AtDCS during the consolidation period and delaying the performance testing when M1 excitability changes are no longer observed. Meanwhile, in the present study we concurrently found a consolidation boosting and an absence of modulations of the following trained sequential series execution times. As well, no effects were found on random series. This picture suggests that AtDCS applied during the consolidation period specifically enhances consolidation, ameliorating the acquired motor engram (retrograde effects, consolidation) instead of affecting subsequent performance ability (anterograde effects).

We included a declarative task as a control condition to check the specificity of the AtDCS effects on procedural memory. Results confirmed the absence of any modulation of declarative memory. Recent data proved that it is possible to interact with procedural learning through proper declarative tasks (Brown and Robertson 2007a,b). Compared with the Brown and Robertson (2007a) task setting, our declarative task is probably too simple and too short to significantly modify procedural consolidation processes. Meanwhile, our subjects were not able to recall more than three to four elements of the nine-element series and all investigated processes occurred over wake with no sleep interaction, since our experiment was performed in a single session. Thus the results of Brown and Robertson (2007a) would lead to the expectation of a block of procedural consolidation induced by the declarative task. On the contrary, we observed an enhancement of consolidation. We could speculate that M1 AtDCS was able to overcome the disrupting action, if any, of our declarative task.

Timing of AtDCS

It could be argued that the timing of neuromodulation application is crucial for the effect on motor learning, since different effects were observed by providing M1 AtDCS before or after the test motor task.

Kuo and colleagues (2008) observed that M1 excitability-enhancing anodal tDCS applied *before* performance reduced motor learning. On the other hand, the application of AtDCS *after* practice, when cortical networks are already selected by the training, provides an enhancement of off-line learning (Boggio et al. 2006; present data). The hypothesis we draw in this frame is that increasing cortical excitability *before* training can facilitate unspecific neuronal recruitments, improving motor performances—instead of procedural learning—the stimulation *after* the training, i.e., when the training session has finalized functional selection of synapses, does strengthen those that enhance consolidation. However, high variability of tasks used to check the tDCS effects requires caution in drawing any conclusive statement about timing of tDCS intervention with respect to the test motor task. In particular, ad hoc paradigms are required to solidly assess whether tDCS should be performed after task execution to optimize consolidation of simple finger sequence movements.

Timing of consolidation (off-line learning)

Our results emphasize previous evidence about the relevance of the early rest period following motor practice in retaining and enhancing procedural learning (Muellbacher et al. 2002; Robertson et al. 2005; Walker et al. 2003). Specifically, these previous experiments (Muellbacher et al. 2002; Robertson et al. 2005) showed that only the early period following the training phase is crucial for retention of improvements, whereas disrupting interventions applied $\geq$2 h later do not affect consolidation processes. Opposite effects on early consolidation could be induced by inhibiting (Muellbacher et al. 2002; Robertson et al. 2005) or enhancing (present data) primary motor cortex excitability, thus supporting the relevance of M1 excitability in consolidation processes of finger movement sequences (Karni et al. 1995).

M1 excitability and learning

Recent advances in understanding the mechanisms of procedural learning confirmed that M1 is an important site for storage of motor sequences (Ashe et al. 2006; Rioult-Pedotti et al. 1998, 2000a,b), with its output organization rapidly modified during learning (Pascual-Leone et al. 1995). In particular, the increase of M1 excitability via AtDCS improved

implicit motor learning of a serial reaction time task (SRTT; Nitsche et al. 2003) and it increased visuomotor coordination learning in humans if applied on primary motor and visual cortices (Antal et al. 2004). Present data corroborate the decisive role of motor cortex excitability in procedural learning (Antal et al. 2004; Muellbacher et al. 2002; Nitsche et al. 2003) and consolidation (Robertson et al. 2005). The positioning of the 5-cm large cephalic electrode (Cogiamanian et al. 2007), centered on C4 and crossing the central sulcus, mostly overlaid primary motor and primary sensory areas. As well, effects on premotor areas cannot strictly be excluded, both as induced by direct stimulation or as an effect of excitability modulations induced in areas connected to the stimulated ones, contributing to consolidation enhancement (Lang et al. 2005; Loubinoux et al. 2001).

In conclusion, our results demonstrated that anodal tDCS applied on M1 soon after training improved early consolidation of procedural learning. We believe that our data highlight the importance of neuromodulation procedures for understanding learning processes and certainly support their use in a motor rehabilitation setting (Boggio et al. 2007; Hummel and Cohen 2005; Schlaug and Renga 2008). To this issue the relevance of tDCS application timing was clearly evidenced.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES

Antal A, Nitsche MA, Kruse W, Kincses TZ, Hoffmann KP, Paulus W. Direct current stimulation over V5 enhances visuomotor coordination by improving motion perception in humans. *J Cogn Neurosci* 16: 521–527, 2004.

Ashe J, Lungu OV, Basford AT, Lu X. Cortical control of motor sequences. *Curr Opin Neurobiol* 16: 213–221, 2006.

Boggio PS, Castro LO, Savagim EA, Braite R, Cruz VC, Rocha RR, Rigonatti SP, Silva MT, Fregni F. Enhancement of non-dominant hand motor function by anodal transcranial direct current stimulation. *Neurosci Lett* 404: 232–236, 2006.

Boggio PS, Nunes A, Rigonatti SP, Nitsche MA, Pascual-Leone A, Fregni F. Repeated sessions of noninvasive brain DC stimulation is associated with motor function improvement in stroke patients. *Restor Neurol Neurosci* 25: 123–129, 2007.

Brashers-Krug T, Shadmehr R, Bizzi E. Consolidation in human motor memory. *Nature* 382: 252–255, 1996.

Broussard DM, Kassardjian CD. Learning in a simple motor system. *Learn Mem* 11: 127–136, 2004.

Brown RM, Robertson EM. Off-line processing: reciprocal interactions between declarative and procedural memories. *J Neurosci* 27: 10468–10475, 2007a.

Brown RM, Robertson EM. Inducing motor skill improvements with a declarative task. *Nat Neurosci* 10: 148–149, 2007b.

Cogiamanian F, Marceglia S, Ardolino G, Barbieri S, Priori A. Improved isometric force endurance after transcranial direct current stimulation over the human motor cortical areas. *Eur J Neurosci* 26: 242–249, 2007.

Hotermans C, Peigneux P, Maertens de Noordhout A, Moonen G, Maquet P. Early boost and slow consolidation in motor skill learning. *Learn Mem* 13: 580–583, 2006.

Hummel F, Cohen LG. Improvement of motor function with noninvasive cortical stimulation in a patient with chronic stroke. *Neurorehabil Neural Repair* 19: 14–19, 2005.

James W. *The Principles of Psychology*. New York: Holt, 1890.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. *Nature* 377: 155–158, 1995.

Karni A, Meyer G, Rey-Hipolity C, Jezzard P, Adamas MM, Turner R, Ungerleider LG. The acquisition of skilled motor performance: fast and slow experience-driven changes in primary motor cortex. *Proc Natl Acad Sci USA* 95: 861–866, 1998.

Krakauer JW, Ghez C, Ghilardi MF. Adaptation to visuomotor transformations: consolidation, interference, and forgetting. *J Neurosci* 25: 473–478, 2005.

Krakauer JW, Shadmehr R. Consolidation of motor memory. *Trends Neurosci* 29: 58–64, 2006.

Kuo MF, Unger M, Liebetanz D, Lang N, Tergau F, Paulus W, Nitsche MA. Limited impact of homeostatic plasticity on motor learning in humans. *Neuropsychologia* 46: 2122–2128, 2005.

Lang N, Siebner HR, Ward NS, Lee L, Nitsche MA, Paulus W, Rothwell JC, Lemon RN, Frackowiak RS. How does transcranial DC stimulation of the primary motor cortex alter regional neuronal activity in the human brain? *Eur J Neurosci* 22: 495–504, 2005.

Liepert J, Classen J, Cohen LG, Hallett M. Task-dependent changes of intracortical inhibition. *Exp Brain Res* 118: 421–426, 1998.

Loubinoux I, Carel C, Alary F, Boulanouar K, Viallard G, Manelfe C, Rascol O, Celsis P, Chollet F. Within-session and between-session reproducibility of cerebral sensorimotor activation: a test–retest effect evidenced with functional magnetic resonance imaging. *J Cereb Blood Flow Metab* 21: 592–607, 2001.

Luft AR, Buitrago MM. Stages of motor skill learning. *Mol Neurobiol* 32: 205–216, 2005.

Muellbacher W, Ziemann U, Boroojerdi B, Cohen L, Hallett M. Role of the human motor cortex in rapid motor learning. *Exp Brain Res* 136: 431–438, 2001.

Muellbacher W, Ziemann U, Wissel J, Dang N, Kofler M, Facchini S, Boroojerdi B, Poewe W, Hallett M. Early consolidation in human primary motor cortex. *Nature* 415: 640–644, 2002.

Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J Physiol* 15: 633–639, 2000.

Nitsche MA, Paulus W. Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology* 57: 1899–1901, 2001.

Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, Tergau F. Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *J Cogn Neurosci* 15: 619–26, 2003.

Nudo RJ, Milliken GW, Jenkins WM, Merzenich MM. Use-dependent alterations of movement representations in primary motor cortex of adult squirrel monkeys. *J Neurosci* 16: 785–807, 1996.

Pascual-Leone A, Nguyet D, Cohen LG, Brasil-Neto JP, Cammarota A, Hallett M. Modulation of muscle responses evoked by transcranial magnetic stimulation during the acquisition of new fine motor skills. *J Neurophysiol* 74: 1037–1045, 1995.

Perez MA, Wise SP, Willingham DT, Cohen LG. Neurophysiological mechanisms involved in transfer of procedural knowledge. *J Neurosci* 27: 1045–1053, 2007.

Plihal W, Born J. Effect of early and late nocturnal sleep on declarative and procedural memory. *J Cogn Neurosci* 9: 534–547, 1997.

Reis J, Schambra HM, Cohen LG, Buch ER, Fritsch B, Zarahn E, Celnik PA, Krakauer JW. Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proc Natl Acad Sci USA* 106: 1590–1595, 2009.

Richardson AG, Overduin SA, Valero-CabrÉ A, Padoa-Schioppa C, Pascual-Leone A, Bizzi E, Press DZ. Disruption of primary motor cortex before learning impairs memory of movement dynamics. *J Neurosci* 26: 12466–12470, 2006.

Rioult-Pedotti M, Donoghue J. The nature and mechanisms of plasticity. In: *Plasticity in the Human Nervous System*, edited by Boniface S, Ziemann U. Cambridge, UK: Cambridge Univ. Press, 2003, p. 1–25.

Rioult-Pedotti MS, Donoghue JP. Neocortical learning regulates synaptic strength by altering the synaptic modification range. *Soc Neurosci Abstr* 26: 732, 2000a.

Rioult-Pedotti MS, Friedman D, Donoghue JP. Learning-induced LTP in neocortex. *Science* 290: 533–536, 2000b.

Rioult-Pedotti MS, Friedman D, Hess G, Donoghue JP. Strengthening of horizontal cortical connections following skill learning. *Nat Neurosci* 1: 230–234, 1998.

Robertson EM, Pascual-Leone A, Miall RC. Current concepts in procedural consolidation. *Nat Rev Neurosci* 5: 576–582, 2004b.

Robertson EM, Pascual-Leone A, Press DZ. Awareness modifies the skill-learning benefits of sleep. *Curr Biol* 14: 208–212, 2004a.

Robertson EM, Press DZ, Pascual-Leone A. Off-line learning and the primary motor cortex. *J Neurosci* 25: 6372–6378, 2005.

Schlaug G, Renga V. Transcranial direct current stimulation: a noninvasive tool to facilitate stroke recovery. *Expert Rev Med Devices* 5: 759–768, 2008.

Shadmehr R, Holcomb HH. Neural correlates of motor memory consolidation. *Science* 8: 821–825, 1997.

Stickgold R, Hobson JA, Fosse R, Fosse M. Sleep, learning, and dreams: off-line memory reprocessing. *Science* 294: 1052–1057, 2001.

Ungerleider LG, Doyon J, Karni A. Imaging brain plasticity during motor skill learning. *Neurobiol Learn Mem* 78: 553–564, 2002.

Walker MP. A refined model of sleep and the time course of memory formation. *Behav Brain Sci* 28: 51–64, 2005.

Walker MP, Brakefield T, Hobson JA, Stickgold R. Dissociable stages of human memory consolidation and reconsolidation. *Nature* 425: 616–620, 2003.

Walker MP, Brakefield T, Morgan A, Hobson JA, Stickgold R. Practice with sleep makes perfect: sleep-dependent motor skill learning. *Neuron* 35: 205–211, 2002.