Neurobiology of Learning and Memory 164 (2019) 107062

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Acquisition and consolidation of implicit motor learning with physical and mental practice across multiple days of anodal tDCS

Ursula Debarnot[a,*], Rémi Neveu[b], Yvette Samaha[a], Elodie Saruco[a,c], Tadhg Macintyre[d], Aymeric Guillot[a]

[a] *Inter-University Laboratory of Human Movement Biology-EA 7424, University of Lyon, University Claude Bernard Lyon 1, 69 622 Villeurbanne, France*
[b] *Division of Child and Adolescent Psychiatry, University of Geneva, 1200 Geneva, Switzerland*
[c] *Neurologische Universitätsklinik, Bergmannsheil gGmbH, Forschungsgruppe Plastizität, Bürkle-de-la-Camp-Platz 1, 44789 Bochum, Germany*
[d] *Health Research Institute, University of Limerick, V94 T9PX Limerick, Ireland*

ARTICLE INFO

Keywords:
Implicit motor sequence learning
Motor imagery
Brain stimulation
Consolidation

ABSTRACT

Background: Acquisition and consolidation of a new motor skill occurs gradually over long time span. Motor imagery (MI) and brain stimulation have been showed as beneficial approaches that boost motor learning, but little is known about the extent of their combined effects.

Objective: Here, we aimed to investigate, for the first time, whether delivering multiple sessions of transcranial direct current stimulation (tDCS) over primary motor cortex during physical and MI practice might improve implicit motor sequence learning in a young population.

Methods: Participants practiced a serial reaction time task (SRTT) either physically or through MI, and concomitantly received either an anodal (excitatory) or sham stimulation over the primary motor cortex during three successive days. The effect of anodal tDCS on the general motor skill and sequence specific learning were assessed on both acquisition (within-day) and consolidation (between-day) processes. We further compared the magnitude of motor learning reached after a single and three daily sessions of tDCS.

Results: The main finding showed that anodal tDCS boosted MI practice, but not physical practice, during the first acquisition session. A second major result showed that compared to sham stimulation, multiple daily session of anodal tDCS, for both types of practice, resulted in greater implicit motor sequence learning rather than a single session of stimulation.

Conclusions: The present study is of particular importance in the context of rehabilitation, where we postulate that scheduling mental training when patients are not able to perform physical movement might beneficiate from concomitant and consecutive brain stimulation sessions over M_1 to promote functional recovery.

1. Introduction

Many of our daily tasks require to acquire and execute sequential complex motor skills, without conscious effort. Learning a new motor sequence implicitly is a process which fundamentally requires repetitive practice of a task, whereby an elaborated memory trace is subsequently consolidated (Ashe, Lungu, Basford, & Lu, 2006; Doyon, 2008). Improving motor learning by targeting acquisition and consolidation processes is one of current challenges in both skill acquisition and functional rehabilitation domains. To date, evidence has accumulated from experimental studies that non-invasive transcranial direct current stimulation technique (tDCS), as well as motor imagery (mental rehearsal of motor task; Jeannerod, 1995), are beneficial approaches promoting implicit motor sequence learning (Kraeutner, MacKenzie, Westwood, & Boe, 2016; Nitsche et al., 2003). Interestingly, implicit motor sequence learning is associated with neuroplastic changes within different cerebral regions (e.g. anterior cingulate cortex, visual cortex, supplementary motor area, dorsolateral prefrontal cortex, Aizenstein et al., 2004; Bischoff-Grethe, Goedert, Willingham, & Grafton, 2004; Pascual-Leone, Wassermann, Grafman, & Hallett, 1996), and is also supported by modulation of activity within the cortico-cerebellar and cortico-striatal systems (Honda et al., 1998; Penhune & Steele, 2012). Most of the studies using the serial reaction time task (SRTT) reported the involvement of the primary motor cortex (M_1, Kantak, Mummidisetty, & Stinear, 2012; Robertson, Press, & Pascual-Leone, 2005) which can be further modulated by both tDCS and MI techniques.

---

* Corresponding author.
E-mail address: Ursula.debarnot@univ-lyon1.fr (U. Debarnot).

https://doi.org/10.1016/j.nlm.2019.107062
Received 18 December 2018; Received in revised form 16 July 2019; Accepted 30 July 2019
Available online 01 August 2019

Yet, however, there is a paucity of knowledge about their combined effects on implicit motor sequence acquisition and consolidation processes across successive days.

Implicit motor sequence learning refers to incidentally improved performance of a motor sequence without overt information about the elements of this sequence (Abrahamse, Jimenez, Verwey, & Clegg, 2010). The general motor skill (GMS) and the sequence-specific (SS) are distinguishable components that contribute to implicit motor sequence learning. While the former refers to faster movements following practice, the latter corresponds to faster movements as a result of the acquired sequence-specific knowledge (Meier & Cock, 2014; Song, Howard, & Howard, 2007). The SRTT (Nissen & Bullemer, 1987) is the most widely used paradigm to assess implicit motor sequence learning, during which a series of stimulus-response pairs, from either a repeated sequence or random events, is presented. In addition to the acquisition of implicit skills that occurs during "online" practice, the memory trace then continues to be processed "offline", without further training (Robertson, 2009). Accordingly, both Nemeth et al. (2010) and Meier and Cock (2014) reported that the GMS improved after a consolidation period ranging from 12 h to 1-week interval, while SS learning only stabilized. Assuming that implicit motor sequence learning in real-life settings occurs gradually over a longer time span, Saevland and Norman (2016) tested the effect of multiple daily sessions of implicit motor sequence learning using a web-based setup. They reported that multiple sessions of practice, rather than a single one, led to accumulated improvements. Despite this promising finding, how both GMS and SS learning might specifically consolidate remains unknown.

Transcranial direct current stimulation (tDCS) has been extensively applied in patients and healthy volunteers, offering the attractive option to modulate neuronal plasticity, and has been found to improve motor learning processes across different phases (Hummel & Cohen, 2006; Nitsche et al., 2008). In delivering a weak current, between an anode over M_1 and a cathode over the contralateral supraorbital area for several minutes, a-tDCS has been shown to increase the cortical excitability for up to 90 min (Nitsche & Paulus, 2001). To date, there is no consensus on both online and offline effects of a-tDCS over M_1 in implicit motor sequence learning. For instance, Nitsche et al. (2003) reported that a-tDCS online improved GMS and SS components of implicit motor learning, while others did not observe such effects in SS learning (Kang and Paik, 2011; Kantak et al., 2012). Similarly, Kang and Paik (2011) reported SS performance gains after consolidation process, while Kantak et al. (2012) only observed a stabilization. In the last decade, there has been a growing interest to deliver a-tDCS multisession protocol first to induce more reliable effect on cortical excitability (Ammann, Lindquist, & Celnik, 2017; Galvez, Alonzo, Martin, & Loo, 2013) and behavioral gains (Fan, Voisin, Milot, Higgins, & Boudrias, 2017; Reis et al., 2009), and second to increase the ecological validity in the clinical and motor learning domains in which beneficial aftereffects seem to outlast tDCS interventions (Hashemirad, Zoghi, Fitzgerald, & Jaberzadeh, 2016; Meinzer, Lindenberg, Antonenko, Flaisch, & Floel, 2013). Nearly all experiments so far tested the effect of a-tDCS during multiple sessions of explicit procedural learning, and reported accumulated gains in performance day after day, compared to sham stimulation (Reis et al., 2009; Saucedo Marquez, Zhang, Swinnen, Meesen, & Wenderoth, 2013; Schambra et al., 2011). Only one study by Dumel et al. (2016) examined the effect of online a-tDCS on implicit motor sequence learning across five consecutive days with an older adult sample. They reported accumulated gains in performance of SS learning over the successive days compared to sham stimulation, but did not test GMS performance. Therefore, whether a-tDCS during implicit motor sequence learning and across consecutive days improve both GMS and SS learning in young adults remains a working hypothesis awaiting further experimental investigation.

A large corpus of evidence from the motor learning literature suggests that imagining an action through MI is a valuable complement to physical practice (PP) in enhancing cognitive and motor performances (Debarnot, Sperduti, Di Rienzo, & Guillot, 2014). MI is the process of mentally rehearsing a motor act without overt body movement (Jeannerod, 1995). A large amount of research provided evidence that MI and PP of the same movement share several characteristics, at the temporal, behavioral and neural levels (Hetu et al., 2013; Holmes & Collins, 2001). Functional brain imaging studies provided evidence that both executed and imagined goal-directed movements recruited overlapping – though not strictly identical – neural networks. For example, M_1 was found to be activated during MI, but to a lesser extent than during movement execution (Carrillo-de-la-Pena, Galdo-Alvarez, & Lastra-Barreira, 2008; Pelgrims, Michaux, Olivier, & Andres, 2011). Furthermore, many investigations demonstrated that MI practice improved explicit sequential motor learning (Debarnot, Abichou, Kalenzaga, Sperduti, & Piolino, 2015; Debarnot, Clerget, & Olivier, 2011; Lacourse, Turner, Randolph-Orr, Schandler, & Cohen, 2004), and elicited similar consolidation process as PP (Debarnot et al., 2012; Debarnot, Castellani, & Guillot, 2012; Debarnot, Creveaux, Collet, Doyon, & Guillot, 2009; Debarnot, Maley, Rossi, & Guillot, 2010). Recently, Kraeutner and collaborators reported that MI practice also yielded improvement on implicit motor sequence learning (Kraeutner, MacKenzie, et al., 2016; Kraeutner, Gaughan, Eppler, & Boe, 2017). However, in this study, they did not evaluate the specific contribution of the GMS, and SS during online learning and offline processes were not tested. Similarly, there are only two experimental studies investigating the effect of a-tDCS with MI practice on explicit sequential motor learning, supporting that these two approaches applied concomitantly are likely to elicit higher performance gains (Foerster et al., 2013; Saimpont et al., 2016). There is a paucity of research on the effect of MI practice in implicit motor sequence learning, compared to that with PP, and less is known about the effect of delivering a-tDCS concomitantly with MI practice.

In light of the above evidence, the present experiment aimed to investigate, for the first time, whether delivering multiple sessions of a-tDCS over M_1 during physical and MI practice might improve implicit motor sequence learning in a young population. Knowing that MI practice is increasingly used as a beneficial adjunctive method to PP in sport and clinical contexts, this study sought to test whether implicit motor sequence learning with MI might elicit similar acquisition and consolidation effects, such as extensively demonstrated with PP. The effects of a-tDCS combined with MI or PP were assessed on both GMS and SS components of implicit learning within (online) and between (offline) each practice session, during three consecutive days. Although the effect of a-tDCS on implicit motor sequence learning is still emerging, we hypothesized that both GMS and SS learning would significantly improve during online and offline processes when a-tDCS was delivered concomitantly with physical and MI practice, rather than with sham stimulation.

2. Methods

2.1. Participants

Forty-eight healthy adults voluntarily participated in this double-blind study. They were randomly assigned to one of the four experimental groups with respect to the type of stimulation ($_{\text{STIM}}$ vs $_{\text{SHAM}}$) and nature of practice (MI vs PP): PP_{SHAM} (mean age 23.16 ± 4.32 years; 7 women), PP_{STIM} (21.50 ± 1.24 years; 7 women), MI_{SHAM} (21.80 ± 3.55 years; 6 women) and MI_{STIM} (22.50 ± 2.94 years; 6 women). All were right-handed, as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). Prior history of drug or alcohol abuse, neurological, musculoskeletal, psychiatric or sleep disorders, constituted exclusion criteria. Participants were considered good sleepers according to the Pittsburgh Sleep Quality Index (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) and provided written informed consent form in agreement with the terms of the declaration of Helsinki. They were individually told about the course of the experiment, and warned about the possible side effects of the stimulation such as heating and/or tingling sensations under the electrodes.

2.2. Experimental procedure

Participants' ability to form vivid and accurate mental images was assessed with the third revised version of the Movement Imagery Questionnaire (MIQ-3; Williams et al., 2012). This 12-item questionnaire involves 4 different movements that participants actually perform and imagine using internal visual, external visual, and kinesthetic imagery. Afterwards, they rated on a 7-point Likert-scale (1: very hard to see/feel and 7: very easy to see/feel) the ease with which they experienced vivid images or intense sensations during each simulated movement. Participants' alertness was also assessed before and after the completion of each experimental session, using the Standford Sleepiness Score questionnaire (Hoddes, Dement, & Zarcone, 1972). As we explored the effect of consolidation following nights of sleep, quality of sleep was controlled by asking to the participants how many hours they slept during the previous night, and whether there suffered from sleep interruptions.

After been randomly assigned into one of the four experimental groups (PP_{SHAM}, PP_{STIM} MI_{SHAM}, MI_{STIM}), all participants attended one experimental session per day, over three consecutive days. Daily sessions were organized into three phases (Fig. 1).

(1) ***Pre-test session***. The pre-test included a sequential practice block (S) during which the implicit 12-items sequence was repeated 5 times (60 trials) and sandwiched between 2 random (R) blocks (60 trials per block).

(2) ***Training session***. Participants from the PP (PP_{SHAM} and PP_{STIM}) and MI groups (MI_{SHAM} and MI_{STIM}) were respectively asked to physically or mentally perform the SRTT during 4 S blocks including 8 sequences (a total of 96 trials per block). To ensure the implicitness of learning, a R block was performed between the 2nd and 3rd S blocks (Kang & Paik, 2011). Participants from the MI groups were asked to imagine themselves performing the motor sequence using a combination of internal visual imagery and kinesthetic imagery, i.e. imagining movement from within one's body and perceiving the sensations usually induced while executing the sequence. At the end of the training, participants in the MI groups reported, on a 6-point Likert-scale, the level of MI vividness and ease experienced to form mental image of the movement (6 being the most vivid and easy score). Concomitantly to PP and MI training, a-tDCS was delivered over M_1 for the PP_{STIM} and MI_{STIM} groups, while sham stimulation

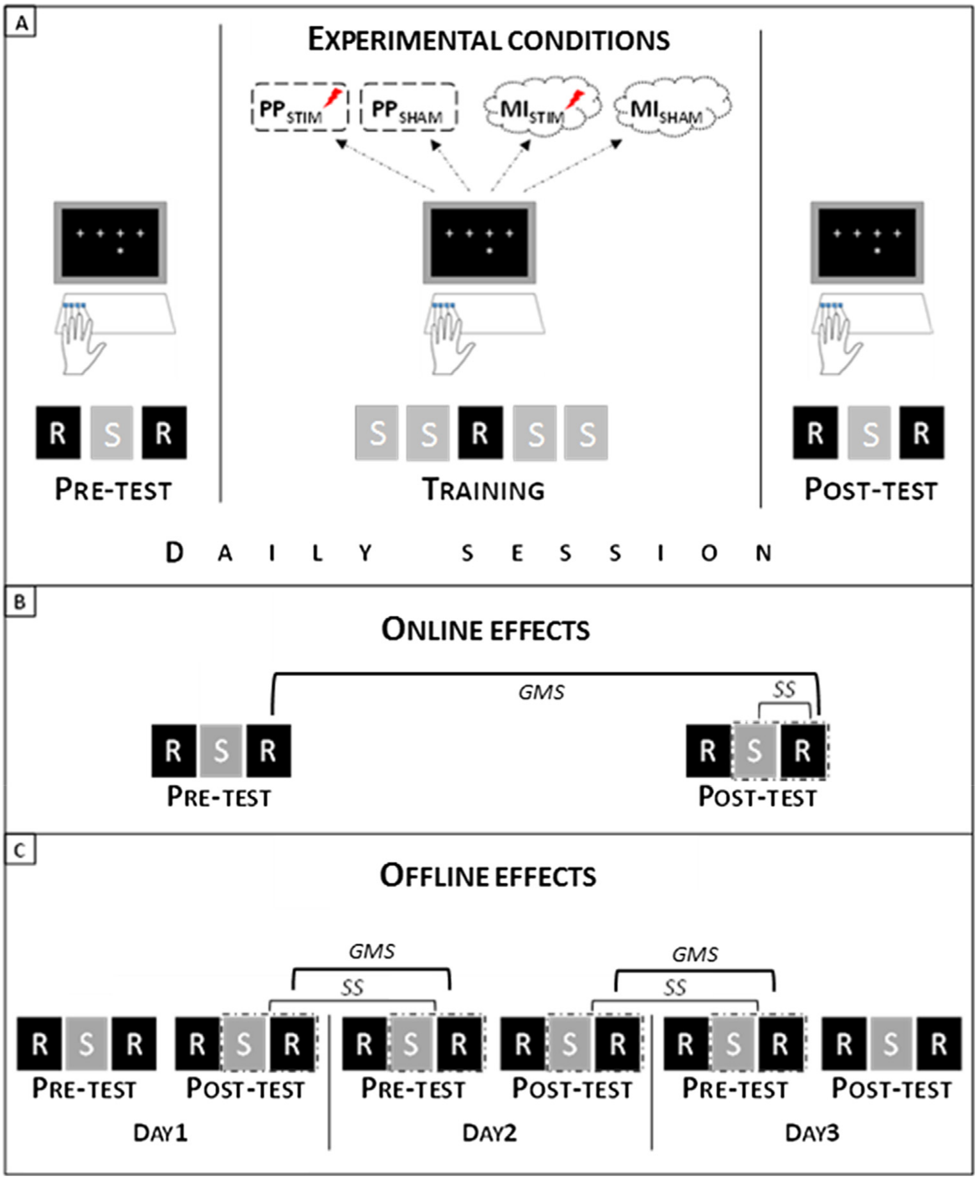

Fig. 1. (A) Experimental design. Participants performed S and R blocks during both testing and training phases along 3 consecutive days. (B) Online effect assessments of implicit motor sequence learning. GMS was assessed by comparing the mean RTs of the random pre-test block with that of the post-test, within each single experimental day. SS was evaluated by subtracting the mean RTs of the post-test random block with the preceding sequential. (C) Offline effects assessments of implicit motor sequence learning. GMS consolidation between days were examined by comparing the mean RTs of random block of the pre-test day 2 to that of the post-test day 1; SS consolidation was investigated by comparing the SS pre-test of the day 2 with that of the day 1 post-test; similar computation has been performed between days 2 and 3. Key: GMS, general motor skill; S, sequence; SS, sequence specific; R, random.

over the same area was delivered for the PP_{SHAM} and MI_{SHAM} groups. Importantly, we used a double-blind approach in which participant and experimenter were blind about the stimulation type.

(3) ***Post-test session***. Immediately after training, participants were subjected to a post-test, which was strictly similar to the pre-test, where a S block (S_{POST}) was sandwiched between 2 R blocks.

At the end of each experimental daily session, and in order to ensure that learning remained implicit, the experimenter asked the same question to each participants: “do you have any comments or remarks about the task or about the way to performed the task?”. At the end of the third daily session, participants' awareness about the sequence repetition was formally examined by a question: “did you notice a repeated pattern of movement during the task?”. Based on these verbal reports, participants were excluded from further analysis when they reported that they perceived a pattern of movement during practice, and when they correctly reported at least four consecutive sequence elements on the keyboard, which were reported on a paper sheet by the experimenter.

2.3. SRTT

A custom SRTT was displayed through MATLAB R2009b (The Mathworks, Inc., Natick, MA, USA), using the Cogent2000v1.32 toolbox. Participants comfortably sat on a chair with their eyes at a distance of 50 cm from the computer screen. Four 1 × 1 cm white crosses aligned horizontally at 6 cm of distance from each other were displayed on a black background screen. On each trial, a 1 × 1 cm white asterisk appeared under one of those crosses. With the little, ring, middle, and index finger of their non-dominant left hand placed respectively over the “Q”, “S”, “D”, and “F” keys of an AZERTY keyboard, participants were asked to press as fast and accurately as possible the key corresponding to the position of the asterisk. In case of incorrect response, the color of the asterisk changed from white to red, and participants were not allowed to correct the movement. The response was also incorrect when participants did not press any key within a 1200 ms delay following the asterisk apparition. During both pre- and post-tests, the asterisk remained on the screen until the participant pressed a key or after a maximum time of 1200 ms. During PP and MI training, the asterisk remained displayed for 1200 ms so each form of training lasted the same amount of time (i.e. a total of 13 min). The asterisk occurrence corresponded to a 12-item sequence that respected the following criteria: (1) the asterisk could not appear under the same cross two consecutive times, (2) the asterisk appeared under each cross an equal number of times, and (3) each possible transition from a location to another occurred only once. To assure that motor learning remained implicit in nature, participants practiced a new sequence each day. The three sequences were similar to those practiced in the study of Dumel et al. (2016; “1-2-4-3-1-3-2-1-4-2-3-4”, “2-3-2-4-1-3-1-4-3-4-2-1” and “4-3-2-4-2-3-1-2-1-4-1-3”), and were counterbalanced across participants and randomized across sessions.

2.4. Stimulation parameters

During training, a-tDCS (STARSTIM, Neuroelectrics) was delivered through two saline-soaked sponge electrodes, an anode ($25\,cm^2$) and a cathode ($35\,cm^2$). The anode was localized contralaterally over M_1, referred to as C4 according to the international 10–20 system. The cathode was placed over the left supraorbital region, referred to as Fp1. Current ramped up to reach 2 mA during the first 30 s, remained at this intensity for 13 min (training period), and then ramped down during the last 30 s. For a high level of blinding, sham stimulations presented similar up and down current modalities but stopped during the training period. At the end of each session, participants were asked whether they perceived they received a real stimulation and could answer “Yes”, “No”, or “I don't know”.

2.5. Data analysis

2.5.1. Data extracted

For each block, any RT longer than 2.7 SDs from participants' mean for the block type was removed from the analysis (Galea, Albert, Ditye, & Miall, 2010). To analyse the GMS and the SS learning, we followed the standard recommendation by Savic and Meier (2016). GMS and SS were computed within and between each day in order to evaluate (1) the effect of training and stimulation types per day (online), and (2) consolidation (offline) along successive days. To investigate the online GMS learning, we subtracted the mean RTs of the random post-test block with that of the pre-test. To explore the offline effect, GMS performance was examined using the mean RTs of the last random post-test block with that of the pre-test the day after (day 1 vs. day 2, and day 2 vs. day 3). Noteworthy, the first random block of the pre-test was considered as a warm up, while the second included prior SS block, hence caution should be exercise as GMS offline learning might have been influenced by SS practice. We further explored the advantage of multiple sessions of implicit motor learning rather than a single, by comparing GMS performances reached at the end of Day 1 with that of Day 3. To investigate the SS online learning, we subtracted the mean RTs of the second random block performed during post-test with the preceding sequential block. To explore the offline effect, we examined the SS performance at post-test day 1 with that of the pre-test the following day. Finally, we explored the advantage of multiple sessions of implicit motor sequence learning rather than a single, by comparing SS performances reached at the end of Day 1 with that of Day 3.

2.5.2. Statistical analysis

We analysed reaction times (RTs) for correct trials in each sequential and random block using lme4 package (Bates, Maechler, & Bolker, 2013) within R software (v3.2.0; Team, 2014). To first verify that motor performance was equivalent across groups at the beginning of the experiment, we modelled (GLM 0) with the RTs of the sequential block during the pre-test of the day 1 as a function of GROUP (MISHAM, MISTIM, PPSHAM, PPSTIM) and their interaction. Then, to investigate the online effect of GMS learning (Fig. 1B), we modelled (GLM 1) with GMS as a function of GROUP (MISHAM, MISTIM, PPSHAM, PPSTIM) and BLOCK (random pre-test vs. random post-test) and their interaction. To investigate the online effect of SS learning (Fig. 1B), we modelled (GLM 2) with SS as function of GROUP (MISHAM, MISTIM, PPSHAM, PPSTIM) and BLOCK (random post-test vs. sequence post-test), and their interaction in each session. Then, to examine the offline effect on GMS learning (Fig. 1C), we modelled the GMS difference between random post-test block day 1 and random pre-test block day 2 as a function of GROUP and their interaction (GLM 3). We did the same for the difference between day 2 and day 3 (GLM 4). To investigate the offline effect on SS learning (Fig. 1C), we modelled the SS difference between post-test day 1 vs. pre-test day 2 as a function of GROUP and BLOCK, and their interaction (GLM 5). We did the same for the difference between Day 2 and Day 3 (GLM 6). Finally, to examine the GMS performance reached during both single and multiple sessions, we modelled the GMS difference between random post-test day 1 and random post-test day 3 as a function of GROUP and their interaction (GLM 7). In the same way, we examined the SS performance reached during both a single and multiple sessions, and modelled the difference between the sequence block at post-test day 1 with that of the post-test day 3 as function of GROUP, and their interaction (GLM 8). When appropriate, corrected Bonferroni post-hoc comparisons were performed. Noteworthy, within group data for both GMS and SS performance per day (acquisition), between days (consolidation) and in the comparison between a single vs. multiple a-tDCS sessions, have been reported in the supplementary file. Scores on questionnaires were compared using ANOVARM. Results are reported as mean ± *SD*, and threshold for significance was set at $P < .05$.

3. Results

3.1. Questionnaires

There was a main session effect before pre-test and after post-test in the Stanford Sleepiness Score ratings ($F_{(5,15)} = 8.19$, $P < .001$), but no group × session interaction ($F_{(3,15)} = 0.06$, $P = .98$). On the 7-point scale (1 = being most alert), mean values for the three experimental days were 2.03 (0.12) before the pre-test, and 2.45 (0.15) after the post-test sessions. With respect to sleep quality, the total sleep time was similar in all participants (8 h ± 1 h), and none reported having sleep trouble during the three considered nights. In regards to individual imagery ability, we did not found a group × mi modality interaction ($F_{(6,88)} = 1.02$, $P = .41$). Therefore, no significant difference was found between MI groups, thus guaranteeing homogeneity in terms of individual ability to form accurate mental images. There was no MI difference when comparing ratings evaluating MI ease ($F_{(2,\ 44)} = 0.58$; $P = .56$), or MI vividness ($F_{(2,\ 44)} = 0.14$; $P = .86$), during each experimental session. Finally, debriefings following MI training revealed that participants used the imagery type given in the instructions, i.e. the first-person perspective. Importantly, three participants were able to recall more than six items of the twelve-item sequence at the end of Day 2, and were therefore excluded from further analyses (one participant in each of the MIsham, MIstim, and PPsham groups).

As far as the blinding of stimulation was concerned in the Sham groups, we performed a Chi-squared test on the proportions of participants' "Yes" and "No" answers for each session, against proportions corresponding to the chance level (50%). Participants who responded "I don't know" were not included in this analysis since they acknowledged not being able to discriminate sham from real stimulations. The conformity tests were not statistically significant (all $P > .05$).

3.2. Online effects

First, we aimed to determine whether the four groups were comparable in terms of performance during the first experimental day. Based on the mean reaction times (RTs) of the pre-test sequential block, subjects from the MIsham, MIstim, PPsham, and PPstim groups respectively performed 520.35 ± 33.36 ms, 522.64 ± 41.11 ms, 511.03 ± 44.04 ms, and 525.59 ± 38.69 ms. The GLM 0 revealed no group difference ($F_{(3,\ 5418)} = 2.05$; $P = .10$), hence ensuring homogeneity across groups.

3.2.1. GMS online learning

To explore the GMS during day 1, we performed the GML 1 that showed a main session effect ($F_{(1,\ 5408)} = 57.20$; $P < .001$), but no group × session interaction ($F_{(3,\ 5408)} = 1.26$; $P = .28$). A different pattern of results was observed on the GMS online performance during day 2. The GLM1 yielded a significant group × session interaction ($F_{(3,\ 5489)} = 3.34$; $P < .05$). Post-hoc analyses revealed a smaller GMS performance in the PPstim compared to all other groups ($P < .05$ vs. MIsham, $P < .01$ vs. MIstim, and $P < .05$ vs. PPsham). Finally, the GML 1 on the GMS of day 3 also showed a group × session interaction ($F_{(3,\ 5203)} = 2.76$; $P < .05$). Post-hoc analyses revealed bigger GMS in the PPsham compared to the PPstim ($P < .05$) and the MIstim ($P < .01$) groups, but not the MIsham group ($P = .40$).

3.2.2. SS online learning

SS learning within each day showed a peculiar pattern of result at day 1 with the MIsham group showing lower performance compared to other groups (Fig. 2). Overall, there was a learning effect within each day independently of the group, excepted for the PPsham at day 3.

The GLM 2 showed a significant group × session interaction ($F_{(3,\ 5474)} = 6.80$; $P < .001$) during Day 1. Post-hoc analyses revealed that the MIsham group showed significant smaller SS learning compared to all other groups ($P < .001$ for all; Fig. 2). Interestingly, no difference was found between the MIstim group compared to PPstim ($P = .56$) and PPsham ($P = .54$) groups, hence attesting that a-tDCS over M_1 during MI practice induced significant learning gains, equivalent to the level of performance reached by PP groups. Moreover, there was no difference between the PPsham and PPstim groups ($P = .18$). Together, these findings clearly indicate that a-tDCS over M_1 significantly boosted SS learning with MI. The GLM 2 on SS learning during day 2 did not reveal a significant group × session interaction ($F_{(3,\ 5166)} = 1.59$; $P = .18$), while the difference reached significance for the day 3 ($F_{(3,\ 41)} = 5.10$; $P < .01$). Post-hoc analysis on the latter interaction however revealed that only the PPsham showed smaller SS learning compared to all other groups ($P < .05$ vs. MIsham and $P < .001$ vs. MIstim and PPstim). As for online SS performance, there was a main effect of block ($P < .001$) each day, hence showing online improvement on the SS learning.

3.3. Offline effects

3.3.1. GMS offline consolidation

All groups showed an effective GMS consolidation between Days 1 and 2, while MIsham learning remained smaller compared to all other groups. The second consolidation period between Days 2 and 3 revealed a bigger learning in the STIM groups relative to MIsham.

Taking into account the difference between mean RTs of the random blocks during the post vs. pre-tests of each day, the GML 3 on GMS performance revealed a significant group × session interaction (day 1 vs. day 2, $F_{(3,\ 5477)} = 3.35$; $P < .05$; day 2 vs. day 3, $F_{(3,\ 5148)} = 4.42$; $P < .01$). Accordingly, post-hoc analyses of the first interaction (day 1 vs. day 2) showed that the MIsham group did not improve GMS learning as much as all other groups ($P < .05$ vs. MIstim, $P < .05$ vs. PPsham, and $P < .01$ vs. PPstim; Fig. 3A). The GML 4 showed quite similar results with the MIsham group demonstrating smaller GMS offline learning compared to both MIstim ($P < .01$) and PPstim ($P < .001$) groups (Fig. 3B). These findings demonstrate GMS learning regardless of the type of practice or the nature of the stimulation between days 1 and 2, while only a-tDCS with MI led to effective improvement, as relative to sham stimulation, between days 2 and 3.

3.3.2. SS offline consolidation

Although, the MI groups stabilized their SS learning during the two consolidation periods (D1 vs D2 and D2 vs D3), the PP groups did not.

When looking at the SS consolidation between days 1 and 2, the GLM 5 revealed a significant group × session × block interaction ($F_{(3,\ 10562)} = 7.28$; $P < .001$). Post-hoc analyses showed that the PPsham group showed smaller SS consolidation compared to all other groups ($P < .001$ with MI groups and $P < .01$ with PPstim; Fig. 4A). Importantly, the MIsham and MIstim groups stabilized their learning between days 1 and 2 ($P = .15$ and $P = .17$, respectively), while PPsham and PPstim groups showed significant smaller SS consolidation during the pre-test of day 2 ($P < .001$ and $P < .01$, respectively). A similar pattern of results was found in the GLM 6 on SS consolidation between days 2 and 3, which yielded a significant group × session × block interaction ($F_{(3,\ 15615)} = 4.18$; $P < .01$; Fig. 4B). Post-hoc analyses revealed that the PPsham group showed smaller SS consolidation compared to MIsham ($P < .01$) and MIstim groups ($P < .01$), who stabilized once again their SS learning ($P = .98$ and $P = .89$, respectively), while there was no difference with the PPstim group ($P < .10$).

3.4. Single vs. multiple daily sessions

3.4.1. GMS effects during single vs. multiple daily sessions

As compared to GMS performance reached after a single session (day 1), all groups protracted further performance gains at the end of the day 3. Nevertheless, the MIsham group showed smaller GMS learning compared to all other groups.

To investigate the potential advantage of delivering multiple successive sessions of practice rather than a single one (Table 3), the GML 7

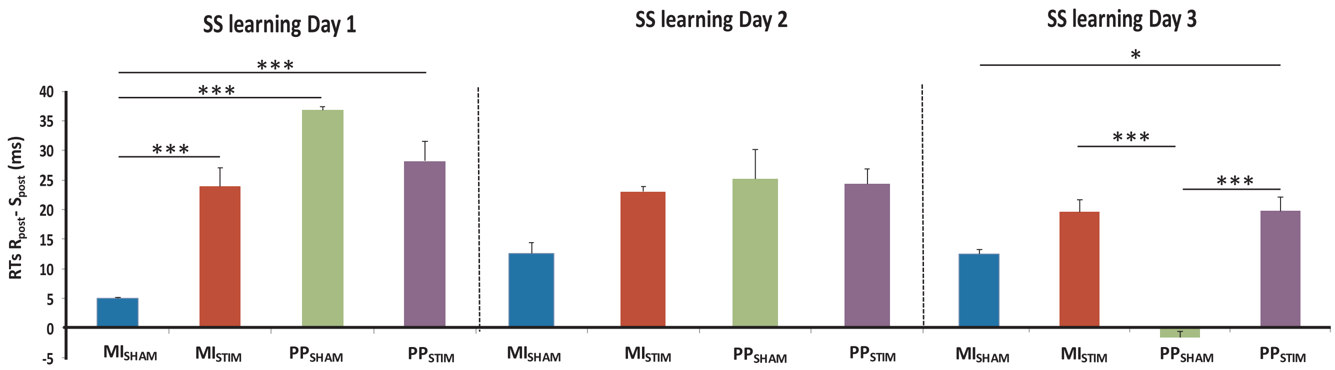

Fig. 2. SS online learning within the three experimental days. Day 1: RTs difference between random and sequence blocks at post-test showed SS performance gains for all groups, while the MISHAM group was significantly slower than all other groups. This data highlights that a-tDCS over M_1 induced substantial performance gains on sequential implicit motor learning with MI, but not PP. Days 2 and 3: there was a general increase in SS learning, while it collapsed at day 3 in the PPSHAM group.

on the GMS component revealed a significant GROUP × SESSION interaction ($F_{(3,\ 5313)} = 8.01$; $P < .001$). Post-hoc analyses revealed that the MISHAM showed less additional GMS performance gains after multiple sessions compared to all other groups ($P < .001$ for all; Fig. 5).

3.4.2. SS effects during single vs. multiple daily sessions

SS learning improved following multiple sessions of practice, with additional benefices observed in the STIM groups.

To investigate the potential advantage of delivering multiple successive sessions of practice rather than a single one (Table 3), the GLM 8 on the SS component revealed a significant GROUP × SESSION interaction ($F_{(3,\ 53553)} = 10.63$; $P < .001$). Post-hoc analyses revealed that the PPSHAM showed less additional SS learning gains after multiple sessions compared to all other groups ($P < .01$ vs. MISHAM, $P < .001$ vs MISTIM and PPSTIM; Fig. 6); albeit there was no difference between the MISHAM and the PPSTIM groups ($P = .19$). This latter data was similar to that of the MISTIM group ($P = .18$) suggesting additional benefits of a-tDCS during multiple session of implicit motor sequence learning.

4. Discussion

The present study was designed to investigate the effect of a-tDCS applied over the right M_1 concomitantly with physical or MI practice of an implicit motor sequence learning, and consolidation over three consecutive days. Data analyses on SS revealed three major findings. First, a-tDCS combined with MI yielded significant SS performance gains during the first day, leading to an equivalent level of performance to that reached with PP. Second, SS consolidation occurred only in the MI groups. Third, multiple daily session of a-tDCS, for both types of practice, resulted in greater SS benefits compared to a single session of stimulation.

4.1. Online effects

GMS learning. All groups improved GMS learning during the first acquisition session, without significant effect of the stimulation, while no further performance gain was observed during subsequent daily sessions of acquisition. This finding challenges previous data showing a significant increase in GMS performance when a-tDCS was administrated over M_1 concomitantly with PP. These contrasting results might be explained by the difference in the quantity of random trials incorporated in the experimental design, which were higher in the present study (Kantak et al., 2012; Nitsche et al., 2003). The young participants enrolled in our experimental design should have reached ceiling GMS performance within the first acquisition session, which restrained room for improvement with a-tDCS. Accordingly, Cooney Horvath, Carter, and Forte (2016) recently showed that a-tDCS over M_1 might not boost simple motor task performance, which echo to the requirement of our random block (i.e. GMS) assessment.

SS learning. A main and original finding revealed that delivering a-tDCS over M_1 concomitantly with MI enhanced the SS performance during the first acquisition session (i.e. day 1), while no difference was observed for PP. The lack of performance gains in the PPSTIM compared to PPSHAM differs from data by Nitsche et al. (2003) and Kantak et al. (2012), who reported improved SS performance following online a-tDCS over M_1 in young healthy participants. Discrepancies may be attributed to the quantity of practice and the stimulation parameters used in the experimental designs. In these two studies, the number of sequential trials were superior to that in the present work (up to 600 vs. 240 trials here), while concomitant a-tDCS intensity was inferior to that of the present work (1 mA vs. 2 mA here). Together, these differences are critical since Miniussi, Harris, and Ruzzoli (2013) argued that behavioral effects of tDCS are the result of an interaction between excitability changes elicited by tDCS and practice. Hence, we postulate that

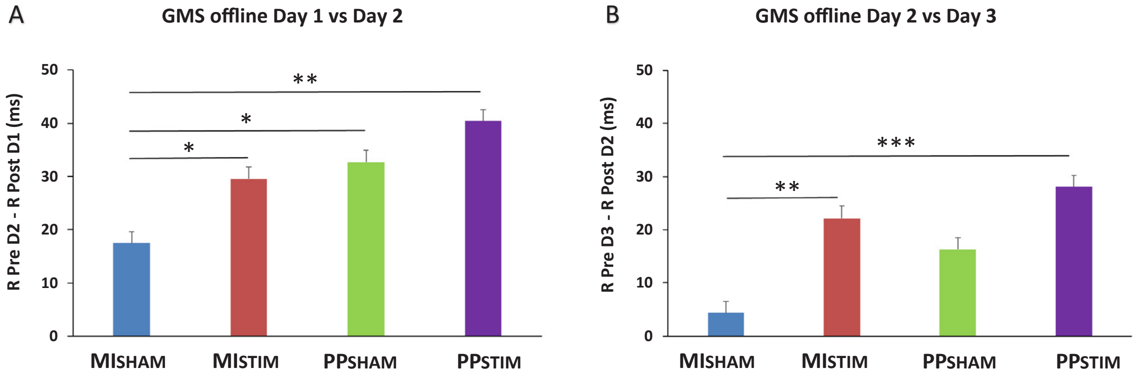

Fig. 3. GMS offline learning. (A) Offline process between days 1 and 2. (B) Offline process between days 2 and 3.

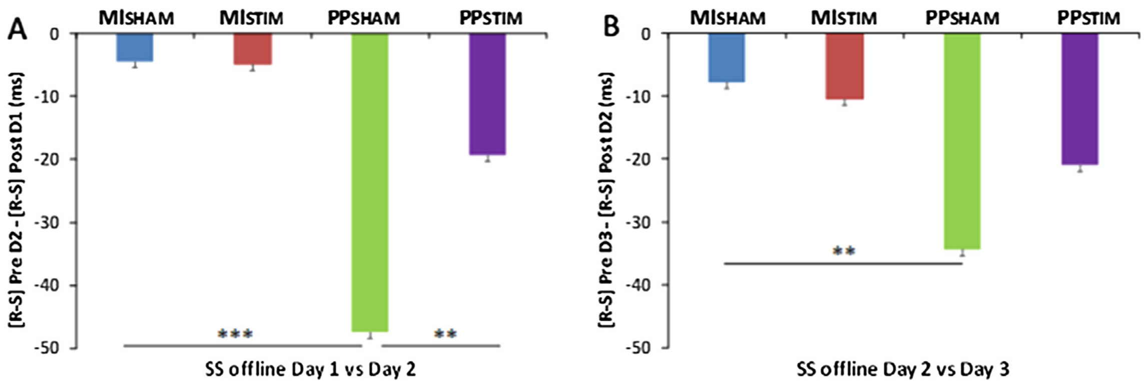

Fig. 4. SS offline consolidation. (A) Offline process between days 1 and 2 showed a collapse in performance in the PPsham group compared to all others groups, while MI groups stabilized their performance. (B) A quite similar pattern of results was observed between days 2 and 3, except that both PP groups yielded an equivalent decrease in SS performance.

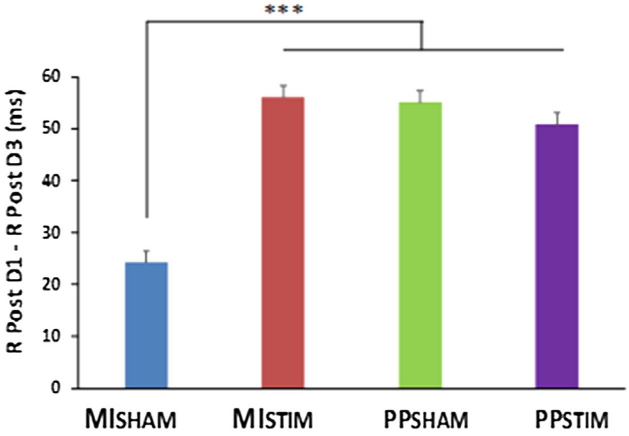

Fig. 5. GMS additional performance gains following multiple sessions of motor learning.

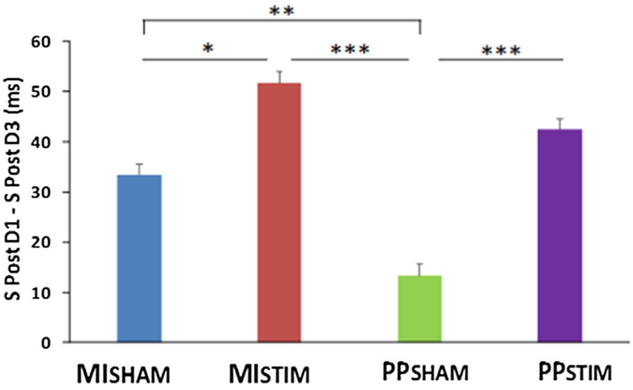

Fig. 6. SS additional performance gains following multiple sessions of motor learning.

application of higher a-tDCS intensity with less motor practice did not trigger significant gains in implicit motor sequence learning, while the reverse protocol is likely to boost it. Future investigations should be conducted to explore the best balance between the amount of motor learning and a-tDCS intensity resulting in significant performance gains. For instance, two groups of subjects might be tested either with low (1 mA) or high (2 mA) a-tDCS intensity over M1, while performing SRTT with less, moderate or extensive amount of trials during three different learning sessions (i.e. using different sequence), separated by a week. A final potential reason for the lack of a-tDCS effect with PP might relate to the proper activation of M_1 during implicit sequential learning. While most of the study have so far reported plastic changes in M_1 using the SRTT (Bischoff-Grethe et al., 2004; Honda et al., 1998; Kantak et al., 2012; Savic & Meier, 2016; Wilkinson, Teo, Obeso, Rothwell, & Jahanshahi, 2010), other showed no effect (Pascual-Leone et al., 1996; Poldrack et al., 2005). Interestingly, our data are in line with recent accumulated evidence of the limited effect of a-tDCS during acquisition of both explicit and implicit motor sequence learning (Bortoletto, Pellicciari, Rodella, & Miniussi, 2015; Kang & Paik, 2011; Vancleef, Meesen, Swinnen, & Fujiyama, 2016), even though the underlying neurophysiological processes that cause such versatility are not yet disentangled. The most salient and novel finding of our study is that a-tDCS during MI practice significantly improved SS performance during the first online acquisition compared to MIsham. This result extends data by Foerster et al. (2013) and Saimpont et al. (2016), who observed comparable benefits of a-tDCS during explicit sequential motor learning with MI. Several studies demonstrated that M_1 is less activated during MI than during PP of the same movement (Carrillo-de-la-Pena et al., 2008; Lotze et al., 1999; Porro et al., 1996). For instance, Lacourse et al. (2004) reported that improvements of motor learning with PP were primarily associated with increased activation in contralateral M_1. The same pattern of results was observed following MI, but to a lesser extent. Hence, it is possible that the susceptibility of M_1 to excitatory effect of a-tDCS is greater during MI training. According to this hypothesis, recent findings showed that MI with excitatory stimulation over M_1 augment corticospinal excitability more than MI alone, and at the level similar to that observed with PP (Kaneko, Hayami, Aoyama, & Kizuka, 2014). Moreover, Moliadze, Antal, and Paulus (2010) recently showed that a low stimulation over the cathode impacted the electric field distribution across the entire cortex, as well as directly under the anode. In the present study, the cathode was positioned over the left frontopolar cortex, which is known to play a critical role in inhibitory processes (Angelini et al., 2015), and during sequential motor learning with MI (Jackson, Lafleur, Malouin, Richards, & Doyon, 2003). Therefore, the effective effect of a-tDCS during MI might be related either to proper activation over right M_1, and/or by a concurrent stimulation over the contralateral frontopolar cortex. Finally, findings also showed that PPsham elicited significant SS performance gains, while MIsham did not. So far, only Kraeutner, MacKenzie, et al. (2016) tested the effect of MI practice in implicit motor sequence learning. They reported equivalent SS performance between MI and PP. However, Kraeutner, MacKenzie, et al. (2016) used a 10-items sequence that was auditory cued, instead of classical 12-items sequence visually cued, before performing either MI or PP movements. Besides these methodological differences, this last finding is in line with numerous investigations demonstrating the remaining advantage to physically rather than mentally perform a movement (Feltz, Landers, & Becker, 1988). Overall, these findings shed light on the optimal parameters of tDCS, in supporting that 13 min of a-tDCS at 2 mA is likely to induce effective performance gains during the acquisition of implicit motor sequence learning with MI, while no further gain was found with PP.

4.2. Offline effects

GMS consolidation. Our data showed that GMS learning improved across consolidation, with significant delayed benefits in combining a-tDCS with MI between days 2 and 3. First, these findings confirm that GMS with PP improved after consolidation periods, as previously reported by Nemeth et al. (2010) and Meier and Cock (2014). Here, and for the first time, our data also showed a delayed benefit induced by a-tDCS on the consolidation of GMS with MI learning. This findings might be due to a ceiling effect of performance reached within the first acquisition session which was not replicated during the second acquisition session, hence allowing a positive a-tDCS effect during the second consolidation period in the MIstim group only. Again, it might be possible that the well-known lower M_1 activation during MI might have resulted in higher sensibility to the a-tDCS compared to PP (Carrillo-de-la-Pena et al., 2008; Lacourse et al., 2004), hence protracting enhanced consolidation performance gain to that reached with PP.

SS consolidation. The study findings revealed that SS consolidation occurred after MI training but not after PP, regardless of the type of stimulation. We introduced a new 12-item sequence (albeit equivalent in complexity, see Section 2.3) for each testing session, to avoid the emergence of explicit acquisition of the sequential movement. Hence, it is possible that the novelty of the implicit sequence might have “interfered” or “hindered” the SS consolidation process following PP. Debarnot et al. (2010) reported that performing a novel interfering motor sequence prevented the expression of delayed gains at 24 h post-training after PP, but not after MI acquisition. Present findings therefore confirm the effector-independent nature of MI practice (Wohldmann, Healy, & Bourne, 2008), and further demonstrate its impermeability to physical retroactive interference. An alternative explanation comes from findings by Breton and Robertson (Breton & Robertson, 2017), who recently reported that offline improvements following the SRTT depend upon a circuit including the left inferior parietal lobule (IPL), but not M_1. Using the same task, Kraeutner, Keeler, and Boe (2016) showed the key role of the left IPL in SS learning with MI. Taken together, these findings highlight the benefits of MI practice, which elicits greater activation of the IPL compared to PP (Lebon, Horn, Domin, & Lotze, 2018), resulting in greater consolidation of implicit motor sequence learning. Determining the role of the IPL in the consolidation process of MI practice will be an exciting focus of research in the coming years.

4.2.1. Single vs. multiple a-tDCS sessions on GMS and SS learning

GMS performance in day 3 was higher in all groups, independently of both stimulation and type of practice. By contrast to the benefits of a-tDCS on total SS learning gain, this finding suggests that a-tDCS might protract additional benefits on complex, but not simple, motor tasks. Future investigations examining different complexities and motor tasks might certainly contribute to further illuminate our understanding of the impact of tDCS on motor learning and consolidation.

As expected, and consistent with the tDCS literature, our data showed higher SS performance in PP participants subjected to stimulation following 3-days of a-tDCS (i.e. S post-Day3), compared to that reached after only one session of stimulation (i.e. S post-Day1). These findings are in line with previous work using multiple a-tDCS protocols concomitantly with implicit (Dumel et al., 2016) and explicit sequential motor learning (Fan et al., 2017; Reis et al., 2009; Saucedo Marquez et al., 2013). Importantly, and for the first time, our results further showed significant benefits in SS performance with MI practice and concomitant a-tDCS. This effective gains in SS performance for both PP and MI practice can be explained by a cumulative increase in the cortical excitability in M_1 when a-tDCS sessions at 2 mA were repeated over several days (Alonzo, Brassil, Taylor, Martin, & Loo, 2012), therefore enhancing learning-related synaptic strength (Nitsche et al., 2008). Moreover, it seems that the larger SS learning at the end of the 3-daily session results from the effect of a-tDCS during acquisition, rather than consolidation, even though caution should be made about the potential effect of consolidation that cannot be clearly disentangled. As M_1 is primarily activated during acquisition of SRTT (Ashe et al., 2006), but no more during consolidation that includes a night of sleep (Robertson et al., 2005), it can be postulated that greater SS performance gains at the end of the third a-tDCS session might result from accumulated increase in M_1 activity during online processes of each daily-session. This assumption is consistent with recent findings by Cantarero, Spampinato, Reis, Ajagbe, and Thompson (2015), who reported that the total motor performance gains after three daily sessions of a-tDCS over the cerebellum were mediated during online, rather than offline process. Overall, our findings support the hypothesis stating that higher beneficial effects are induced by consecutive a-tDCS sessions during SS learning, and underline for the first time similar benefits in performance using MI practice. This result is of particular importance in the context of rehabilitation, where we postulate that scheduling MI training when patients are not able to perform physical movement might beneficiate from concomitant and consecutive a-tDCS sessions over M_1 to boost functional recovery.

5. Conclusions

The present study is the first study to specifically target an investigation into the effects of acquisition and consolidation on each component of an implicit motor sequence learning across multiple daily session of physical and MI practice, and further explore the impacts of using adjunctive a-tDCS over M_1. The most striking finding is that a-tDCS boosted MI practice, but not PP, during the first acquisition session. Another important insight is that combining a-tDCS with both types of practice, across three daily sessions, significantly contributed to improve implicit motor sequence learning rather than a single session. Such findings have strong theoretical implications and practical applications in both motor learning and motor rehabilitation areas. Repeated stimulation sessions usually result in long-lasting effects in postsynaptic connections similar to long-term potentiation, a critical process for alleviating motor deficits in patients. Investigating the effect of a-tDCS of imagined movement is still in its infancies, while it is an efficient alternative to PP, which is widely used in the clinical context.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nlm.2019.107062.

References

Abrahamse, E. L., Jimenez, L., Verwey, W. B., & Clegg, B. A. (2010). Representing serial action and perception. *Psychonomic Bulletin & Review, 17*, 603–623.

Aizenstein, H. J., Stenger, V. A., Cochran, J., Clark, K., Johnson, M., Nebes, R. D., & Carter, C. S. (2004). Regional brain activation during concurrent implicit and explicit sequence learning. *Cerebral Cortex, 14*, 199–208.

Alonzo, A., Brassil, J., Taylor, J. L., Martin, D., & Loo, C. K. (2012). Daily transcranial direct current stimulation (tDCS) leads to greater increases in cortical excitability than second daily transcranial direct current stimulation. *Brain Stimulation, 5*, 208–213.

Ammann, C., Lindquist, M. A., & Celnik, P. A. (2017). Response variability of different anodal transcranial direct current stimulation intensities across multiple sessions. *Brain Stimulation, 10*, 757–763.

Angelini, M., Calbi, M., Ferrari, A., Sbriscia-Fioretti, B., Franca, M., Gallese, V., & Umilta, M. A. (2015). Motor inhibition during overt and covert actions: An electrical neuroimaging study. *PLoS ONE, 10*, e0126800.

Ashe, J., Lungu, O. V., Basford, A. T., & Lu, X. (2006). Cortical control of motor sequences. *Current Opinion in Neurobiology, 16*, 213–221.

Bates, D., Maechler, M., & Bolker, B. (2013). lme4: Linear-mixed Effects Models Using S4 Classes. Retrieved from: http://CRAN.R-project.org/package=lme4 (R package version 0.999999-2).

Bischoff-Grethe, A., Goedert, K. M., Willingham, D. T., & Grafton, S. T. (2004). Neural substrates of response-based sequence learning using fMRI. *Journal of Cognitive*

Neuroscience, 16, 127–138.

Bortoletto, M., Pellicciari, M. C., Rodella, C., & Miniussi, C. (2015). The interaction with task-induced activity is more important than polarization: A tDCS study. *Brain Stimulation, 8*, 269–276.

Breton, J., & Robertson, E. M. (2017). Dual enhancement mechanisms for overnight motor memory consolidation. *Nature Human Behaviour, 1*.

Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh Sleep Quality Index: A new instrument for psychiatric practice and research. *Psychiatry Research, 28*, 193–213.

Cantarero, G., Spampinato, D., Reis, J., Ajagbe, L., & Thompson, T. (2015). Cerebellar direct current stimulation enhances on-line motor skill acquisition through an effect on accuracy. *The Journal of Neuroscience, 35*, 3285–3290.

Carrillo-de-la-Pena, M. T., Galdo-Alvarez, S., & Lastra-Barreira, C. (2008). Equivalent is not equal: Primary motor cortex (MI) activation during motor imagery and execution of sequential movements. *Brain Research, 1226*, 134–143.

Debarnot, U., Abichou, K., Kalenzaga, S., Sperduti, M., & Piolino, P. (2015). Variable motor imagery training induces sleep memory consolidation and transfer improvements. *Neurobiology of Learning and Memory, 119*, 85–92.

Debarnot, U., Castellani, E., & Guillot, A. (2012). Selective delayed gains following motor imagery of complex movements. *Archives Italiennes de Biologie, 150*, 238–250.

Debarnot, U., Castellani, E., Guillot, A., Giannotti, V., Dimarco, M., & Sebastiani, L. (2012). Declarative interference affects off-line processing of motor imagery learning during both sleep and wakefulness. *Neurobiology of Learning and Memory, 98*, 361–367.

Debarnot, U., Clerget, E., & Olivier, E. (2011). Role of the primary motor cortex in the early boost in performance following mental imagery training. *PLoS ONE, 6*, e26717.

Debarnot, U., Creveaux, T., Collet, C., Doyon, J., & Guillot, A. (2009). Sleep contribution to motor memory consolidation: A motor imagery study. *Sleep, 32*, 1559–1565.

Debarnot, U., Maley, L., Rossi, D. D., & Guillot, A. (2010). Motor interference does not impair the memory consolidation of imagined movements. *Brain and Cognition, 74*, 52–57.

Debarnot, U., Sperduti, M., Di Rienzo, F., & Guillot, A. (2014). Experts bodies, experts minds: How physical and mental training shape the brain. *Frontiers in Human Neuroscience, 8*, 280.

Doyon, J. (2008). Motor sequence learning and movement disorders. *Current Opinion in Neurology, 21*, 478–483.

Dumel, G., Bourassa, M. E., Desjardins, M., Voarino, N., Charlebois-Plante, C., Doyon, J., & De Beaumont, L. (2016). Multisession anodal tDCS protocol improves motor system function in an aging population. *Neural Plasticity, 2016*, 5961362.

Fan, J., Voisin, J., Milot, M. H., Higgins, J., & Boudrias, M. H. (2017). Transcranial direct current stimulation over multiple days enhances motor performance of a grip task. *Annals of Physical and Rehabilitation Medicine, 60*, 329–333.

Feltz, D., Landers, D. M., & Becker, B. J. (1988). A revised meta-analysis of the mental practice literature on motor skill learning. In D. Druckmann, & J. A. Swets (Eds.). *Enhancing human performance: Issues, theories, and techniques* (pp. 61–101). Washington D.C.: National Academy Press.

Foerster, A., Rocha, S., Wiesiolek, C., Chagas, A. P., Machado, G., Silva, E., ... Monte-Silva, K. (2013). Site-specific effects of mental practice combined with transcranial direct current stimulation on motor learning. *European Journal of Neuroscience, 37*, 786–794.

Galea, J. M., Albert, N. B., Ditye, T., & Miall, R. C. (2010). Disruption of the dorsolateral prefrontal cortex facilitates the consolidation of procedural skills. *Journal of Cognitive Neuroscience, 22*, 1158–1164.

Galvez, V., Alonzo, A., Martin, D., & Loo, C. K. (2013). Transcranial direct current stimulation treatment protocols: Should stimulus intensity be constant or incremental over multiple sessions? *International Journal of Neuropsychopharmacology, 16*, 13–21.

Hashemirad, F., Zoghi, M., Fitzgerald, P. B., & Jaberzadeh, S. (2016). The effect of anodal transcranial direct current stimulation on motor sequence learning in healthy individuals: A systematic review and meta-analysis. *Brain and Cognition, 102*, 1–12.

Hetu, S., Gregoire, M., Saimpont, A., Coll, M. P., Eugene, F., Michon, P. E., & Jackson, P. L. (2013). The neural network of motor imagery: An ALE meta-analysis. *Neuroscience & Biobehavioral Reviews, 37*, 930–949.

Hoddes, E., Dement, W. C., & Zarcone, V. (1972). The development and use of the Stanford sleepiness scale. *Psychophysiology, 9*, 150.

Holmes, P. S., & Collins, D. J. (2001). The PETTLEP approach to motor imagery: A functional equivalence model for sport psychologists. *Journal of Applied Sport Psychology, 13*, 60–83.

Honda, M., Deiber, M. P., Ibanez, V., Pascual-Leone, A., Zhuang, P., & Hallett, M. (1998). Dynamic cortical involvement in implicit and explicit motor sequence learning. A PET study. *Brain, 121*(Pt 11), 2159–2173.

Horvath, J. C., Carter, O., & Forte, J. D. (2016). No significant effect of transcranial direct current stimulation (tDCS) found on simple motor reaction time comparing 15 different simulation protocols. *Neuropsychologia, 91*, 544–552.

Hummel, F. C., & Cohen, L. G. (2006). Non-invasive brain stimulation: A new strategy to improve neurorehabilitation after stroke? *Lancet Neurology, 5*, 708–712.

Jackson, P. L., Lafleur, M. F., Malouin, F., Richards, C. L., & Doyon, J. (2003). Functional cerebral reorganization following motor sequence learning through mental practice with motor imagery. *Neuroimage, 20*, 1171–1180.

Jeannerod, M. (1995). Mental imagery in the motor context. *Neuropsychologia, 33*, 1419–1432.

Kaneko, F., Hayami, T., Aoyama, T., & Kizuka, T. (2014). Motor imagery and electrical stimulation reproduce corticospinal excitability at levels similar to voluntary muscle contraction. *Journal of NeuroEngineering and Rehabilitation, 11*, 94.

Kang, E. K., & Paik, N. J. (2011). Effect of a tDCS electrode montage on implicit motor sequence learning in healthy subjects. *Experimental & Translational Stroke Medicine, 3*, 4.

Kantak, S. S., Mummidisetty, C. K., & Stinear, J. W. (2012). Primary motor and premotor cortex in implicit sequence learning–Evidence for competition between implicit and explicit human motor memory systems. *European Journal of Neuroscience, 36*, 2710–2715.

Kraeutner, S. N., Gaughan, T. C., Eppler, S. N., & Boe, S. G. (2017). Motor imagery-based implicit sequence learning depends on the formation of stimulus-response associations. *Acta Psychologica (Amst), 178*, 48–55.

Kraeutner, S. N., Keeler, L. T., & Boe, S. G. (2016a). Motor imagery-based skill acquisition disrupted following rTMS of the inferior parietal lobule. *Experimental Brain Research, 234*, 397–407.

Kraeutner, S. N., MacKenzie, L. A., Westwood, D. A., & Boe, S. G. (2016b). Characterizing skill acquisition through motor imagery with no prior physical practice. *Journal of Experimental Psychology: Human Perception and Performance, 42*, 257–265.

Lacourse, M. G., Turner, J. A., Randolph-Orr, E., Schandler, S. L., & Cohen, M. J. (2004). Cerebral and cerebellar sensorimotor plasticity following motor imagery-based mental practice of a sequential movement. *Journal of Rehabilitation Research and Development, 41*, 505–524.

Lebon, F., Horn, U., Domin, M., & Lotze, M. (2018). Motor imagery training: Kinesthetic imagery strategy and inferior parietal fMRI activation. *Human Brain Mapping, 39*, 1805–1813.

Lotze, M., Montoya, P., Erb, M., Hulsmann, E., Flor, H., Klose, U., ... Grodd, W. (1999). Activation of cortical and cerebellar motor areas during executed and imagined hand movements: An fMRI study. *Journal of Cognitive Neuroscience, 11*, 491–501.

Meier, B., & Cock, J. (2014). Offline consolidation in implicit sequence learning. *Cortex, 57*, 156–166.

Meinzer, M., Lindenberg, R., Antonenko, D., Flaisch, T., & Floel, A. (2013). Anodal transcranial direct current stimulation temporarily reverses age-associated cognitive decline and functional brain activity changes. *Journal of Neuroscience, 33*, 12470–12478.

Miniussi, C., Harris, J. A., & Ruzzoli, M. (2013). Modelling non-invasive brain stimulation in cognitive neuroscience. *Neuroscience & Biobehavioral Reviews, 37*, 1702–1712.

Moliadze, V., Antal, A., & Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clinical Neurophysiology, 121*, 2165–2171.

Nemeth, D., Janacsek, K., Londe, Z., Ullman, M. T., Howard, D. V., & Howard, J. H., Jr. (2010). Sleep has no critical role in implicit motor sequence learning in young and old adults. *Experimental Brain Research, 201*, 351–358.

Nissen, M. J., & Bullemer, P. (1987). Attentional requirements of learning: Evidence from performance measures. *Cognitive Psychology, 19*, 1–32.

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., ... Pascual-Leone, A. (2008). Transcranial direct current stimulation: State of the art 2008. *Brain Stimulation, 1*, 206–223.

Nitsche, M. A., & Paulus, W. (2001). Sustained excitability elevations induced by transcranial DC motor cortex stimulation in humans. *Neurology, 57*, 1899–1901.

Nitsche, M. A., Schauenburg, A., Lang, N., Liebetanz, D., Exner, C., Paulus, W., & Tergau, F. (2003). Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. *Journal of Cognitive Neuroscience, 15*, 619–626.

Pascual-Leone, A., Wassermann, E. M., Grafman, J., & Hallett, M. (1996). The role of the dorsolateral prefrontal cortex in implicit procedural learning. *Experimental Brain Research, 107*, 479–485.

Pelgrims, B., Michaux, N., Olivier, E., & Andres, M. (2011). Contribution of the primary motor cortex to motor imagery: A subthreshold TMS study. *Human Brain Mapping, 32*, 1471–1482.

Penhune, V. B., & Steele, C. J. (2012). Parallel contributions of cerebellar, striatal and M1 mechanisms to motor sequence learning. *Behavioural Brain Research, 226*, 579–591.

Poldrack, R. A., Sabb, F. W., Foerde, K., Tom, S. M., Asarnow, R. F., Bookheimer, S. Y., & Knowlton, B. J. (2005). The neural correlates of motor skill automaticity. *Journal of Neuroscience, 25*, 5356–5364.

Porro, C. A., Francescato, M. P., Cettolo, V., Diamond, M. E., Baraldi, P., Zuiani, C., ... di Prampero, P. E. (1996). Primary motor and sensory cortex activation during motor performance and motor imagery: A functional magnetic resonance imaging study. *Journal of Neuroscience, 16*, 7688–7698.

Reis, J., Schambra, H. M., Cohen, L. G., Buch, E. R., Fritsch, B., Zarahn, E., ... Krakauer, J. W. (2009). Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation. *Proceedings of the National Academy of Sciences of the United States of America, 106*, 1590–1595.

Robertson, E. M. (2009). From creation to consolidation: A novel framework for memory processing. *PLoS Biology, 7*, e19.

Robertson, E. M., Press, D. Z., & Pascual-Leone, A. (2005). Off-line learning and the primary motor cortex. *Journal of Neuroscience, 25*, 6372–6378.

Saevland, W., & Norman, E. (2016). Studying different tasks of implicit learning across multiple test sessions conducted on the web. *Frontiers in Psychology, 7*, 808.

Saimpont, A., Mercier, C., Malouin, F., Guillot, A., Collet, C., Doyon, J., & Jackson, P. L. (2016). Anodal transcranial direct current stimulation enhances the effects of motor imagery training in a finger tapping task. *European Journal of Neuroscience, 43*, 113–119.

Saucedo Marquez, C. M., Zhang, X., Swinnen, S. P., Meesen, R., & Wenderoth, N. (2013). Task-specific effect of transcranial direct current stimulation on motor learning. *Frontiers in Human Neuroscience, 7*, 333.

Savic, B., & Meier, B. (2016). How transcranial direct current stimulation can modulate implicit motor sequence learning and consolidation: A brief review. *Frontiers in Human Neuroscience, 10*, 26.

Schambra, H. M., Abe, M., Luckenbaugh, D. A., Reis, J., Krakauer, J. W., & Cohen, L. G. (2011). Probing for hemispheric specialization for motor skill learning: A transcranial direct current stimulation study. *Journal of Neurophysiology, 106*, 652–661.

Song, S., Howard, J. H., Jr., & Howard, D. V. (2007). Implicit probabilistic sequence learning is independent of explicit awareness. *Learning & Memory, 14*, 167–176.

Team, R. C. (2014). R: A Language and Environment for Statistical Computing. Vienna: Foundation for Statistical Computing.

Vancleef, K., Meesen, R., Swinnen, S. P., & Fujiyama, H. (2016). tDCS over left M1 or DLPFC does not improve learning of a bimanual coordination task. *Scientific Reports, 6*, 35739.

Wilkinson, L., Teo, J. T., Obeso, I., Rothwell, J. C., & Jahanshahi, M. (2010). The contribution of primary motor cortex is essential for probabilistic implicit sequence learning: Evidence from theta burst magnetic stimulation. *Journal of Cognitive Neuroscience, 22*, 427–436.

Williams, S. E., Cumming, J., Ntoumanis, N., Nordin-Bates, S. M., Ramsey, R., & Hall, C. (2012). Further validation and development of the movement imagery questionnaire. *Journal of Sport and Exercise Psychology, 34*, 621–646.

Wohldmann, E. L., Healy, A. F., & Bourne, L. E. (2008). A mental practice superiority effect: Less retroactive interference and more transfer than physical practice. *Journal of Experimental Psychology. Learning, Memory, and Cognition, 34*, 823–833.