DOES TRANSCRANIAL DIRECT CURRENT STIMULATION AFFECT THE LEARNING OF A FINE SEQUENTIAL HAND MOTOR SKILL WITH MOTOR IMAGERY?

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Abstract

Learning a fine sequential hand motor skill, like playing the piano or learning to type, improves not only due to physical practice, but also due to motor imagery. Previous studies revealed that transcranial direct current stimulation (tDCS) and motor imagery independently affect motor learning. In the present study, we investigated whether tDCS combined with motor imagery above the primary motor cortex influences sequence-specific learning. Four groups of participants were involved: an anodal, cathodal, sham stimulation, and a control group (without stimulation). A modified discrete sequence production (DSP) task was employed: the Go/NoGo DSP task. After a sequence of spatial cues, a response sequence had to be either executed, imagined, or withheld. This task allows to estimate both non-specific learning and sequence-specific learning effects by comparing the execution of unfamiliar sequences, familiar imagined, familiar withheld, and familiar executed sequences in a test phase. Results showed that the effects of anodal tDCS were already developing during the practice phase, while no effect of tDCS on sequence-specific learning were visible during the test phase. Results clearly showed that motor imagery itself influences sequence learning, but we also revealed that tDCS does not increase the influence of motor imagery on sequence learning.

Key words: motor imagery, motor learning, transcranial direct current stimulation (tDCS), Go/NoGo DSP task.

Introduction

Transcranial direct current stimulation (tDCS) is a noninvasive technique that aims to modulate cortical excitability by delivering a weak constant current between two electrodes placed over the scalp. It has been shown that anodal stimulation of the motor cortex enhances cortical excitability, while excitability diminishes in the case of cathodal stimulation (Nitsche & Paulus, 2000; Quartarone, et al., 2004). The common explanation of these effects is that cathodal stimulation induces hyperpolarization of neurons, while anodal stimulation results in depolarization (Nitsche & Paulus, 2000), leading to a decrease or an increase of cerebral excitability, respectively (Bindman, Lippold, & Redfearn, 1964; Purpura & McMurtry, 1965). These cortical changes in excitability due to tDCS can be explained by phenomena like long-term potentiation (LTP) and long-term depression (LTD), (Malenka & Nicoll, 1999). Apart from the type of stimulation (anodal or cathodal), the effects of tDCS depend on the intensity of the stimulation, the precise location of the electrodes, and stimulation duration (Nitsche & Paulus, 2001; Nitsche, et al., 2003; Kaminski, et al., 2013).

It has been shown in previous studies that tDCS may have positive effects on motor skill learning (Antal, Nitsche, Kruse, Hoffmann, & Paulus, 2004; Ciechanski & Kirton, 2016; Buch, et al., 2017). Interestingly, several studies also revealed that motor skills may improve due to motor imagery (defined as the mental simulation of a movement without its actual execution (Jeannerod, 2001)). For example, it has been shown that training with motor imagery has positive effects on motor performance in athletes, musicians and healthy subjects (Driskell, Copper, & Moran, 1994; Pascual-Leone, et al., 1995; Jackson, Lafleur, Malouin, & Richards, 2001; Gentili, Papaxanthis, & Pozzo, 2006; Debarnot, Clerget, & Olivier, 2011). Moreover, it has been revealed that motor imagery improves motor strength (Lebon, Collet, & Guillot, 2010), movement velocity (Pascual-Leone, et al., 1995), and motor recovery (Cho, Kim, & Lee, 2013; Maillet, et al., 2013). However, an important distinction that needs to be made when

considering improved performance is whether the effect can be considered as a non-specific learning effect, which may simply be due to increased task familiarity, or as a sequence-specific learning effect, which relates to learning to carry out a specific sequence of actions (Keele, Ivry, Mayr, Hazeltine, & Heuer, 2003; Verwey & Wright, 2014). In our study we focused on both non-specific and sequence-specific learning effects due to tDCS. Thus, the question may be raised whether tDCS affects sequence-specific learning. Additionally, the combined use of tDCS and motor imagery might boost sequence-specific learning.

Foerster, Rocha, Wiesiolek, Chagas, Machado, Silva, Fregni, and Monte-Silva (2013) examined whether tDCS combined with motor imagery enhances motor performance by using a handwriting test. Their results revealed that anodal tDCS combined with motor imagery significantly reduced the time needed in the handwriting task as compared with sham stimulation. In line with Nitsche and Paulus (2000), Foerster et al. (2013) explained their findings in terms of increased cortical excitability induced by anodal tDCS and mental practice. However, no effect of training with motor imagery was observed, therefore the reduction of handwriting time could also be solely due to anodal tDCS. As the effect of tDCS alone was not examined, this possibility cannot be excluded. The results of Foerster et al. (2013) are partially consistent with the results of a recent study of Saimpont, Mercier, Malouin, Guillot, Collet, Doyon, and Jackson (2016). In that study, it was examined whether anodal tDCS strengthened the effect of motor imagery while learning a finger tapping sequence. Results revealed that anodal tDCS together with motor imagery training significantly increased the number of correct sequences compared with sham stimulation or tDCS alone. Motor imagery training and tDCS alone also significantly improved motor performance (Saimpont, et al., 2016), but the combination of tDCS and motor imagery induced stronger learning effects than each method alone. Saimpont et al. (2016) explained these findings by the reinforcement of synaptic strength within the primary motor cortex. Importantly, both above-mentioned studies focused on rather

general non-specific learning effects instead of sequence-specific learning effects (see: (Sobierajewicz J. , Przekoracka-Krawczyk, Jaśkowski, Verwey, & van der Lubbe, 2017)). Thus, the question remains whether the combination of tDCS and motor imagery also enhances sequence-specific learning of a fine motor skill.

Sequence learning refers to acquiring the skill to produce a sequence of actions as fast and accurately as possible (Keele, Ivry, Mayr, Hazeltine, & Heuer, 2003; Verwey & Wright, 2014). Non-specific learning effects, reflected in improved performance may occur due to multiple factors like increased familiarity with the task procedure or an improved ability to decode stimuli. To establish whether sequence learning effects are not non-specific, control (unfamiliar) sequences should be added to a final test phase. Thus, during practice participants execute particular sequences (either physically or mentally), and in the test phase motor performance of these familiar sequences is compared with unfamiliar sequences. Application of this method revealed that motor execution and motor imagery both induce sequence-specific learning effects (Sobierajewicz, Szarkiewicz, Przekoracka-Krawczyk, Jaśkowski, & van der Lubbe, 2016; Sobierajewicz J. , Przekoracka-Krawczyk, Jaśkowski, Verwey, & van der Lubbe, 2017).

The aim of the current study was twofold. We were interested in establishing whether learning effects of tDCS can be considered as non-specific and/or sequence-specific. Furthermore, we wanted to know whether these effects increase when tDCS is combined with motor imagery. First of all, we expected that tDCS improves motor performance, which can be examined by comparing results between groups of participants that receive anodal or cathodal tDCS or not. In contrast with the above-mentioned studies in which anodal and sham stimulation were compared (Foerster, et al., 2013; Saimpont, et al., 2016), we examined the influence of anodal and cathodal stimulation, and next to sham stimulation we also included a control group. We expected to observe better performance in the anodal tDCS group, and in the

case of cathodal stimulation we expected that the effects of learning would be diminished; while similar results were expected for the sham stimulation and the control group as these groups did not receive any stimulation. Furthermore, by including a control group the possibility of a placebo effect can be ruled out. Secondly, by employing the Go/NoGo Discrete Sequence Production task (see (Sobierajewicz, Szarkiewicz, Przekoracka-Krawczyk, Jaśkowski, & van der Lubbe, 2016; Sobierajewicz J. , Przekoracka-Krawczyk, Jaśkowski, Verwey, & van der Lubbe, 2017)) we can examine the influence of tDCS on sequence-specific learning by comparing performance for familiar sequences (trained in the practice phase) with unfamiliar (untrained) sequences in the final test phase, and examine this difference between groups. Importantly, as we observed that motor imagery induces sequence-specific learning effects (Sobierajewicz et al., 2016, 2017), we also wanted to verify if this effect increases due to the application of tDCS. By comparing the results between familiar imagined and unfamiliar sequences in the test phase and comparing this difference between groups, we might demonstrate that tDCS boosts the effect of motor imagery on sequence-specific learning.

Methods

Participants

Forty-eight volunteers took part in the experiment (34 female, 14 male). All participants reported to have no history of mental and neurological disorders, no family history of epilepsy, cardiac pacemaker or metallic implants. Participants were aged between 20 and 34 years (M_{age} = 24.5, SD 3.7). Prior to the experiment they were asked to sign an informed consent and to complete Annett`s Handedness Inventory (Annett, 1970). Participants were randomly assigned to one of four groups (12 participants in each group): 1) *anodal* – six female, six male, all right-

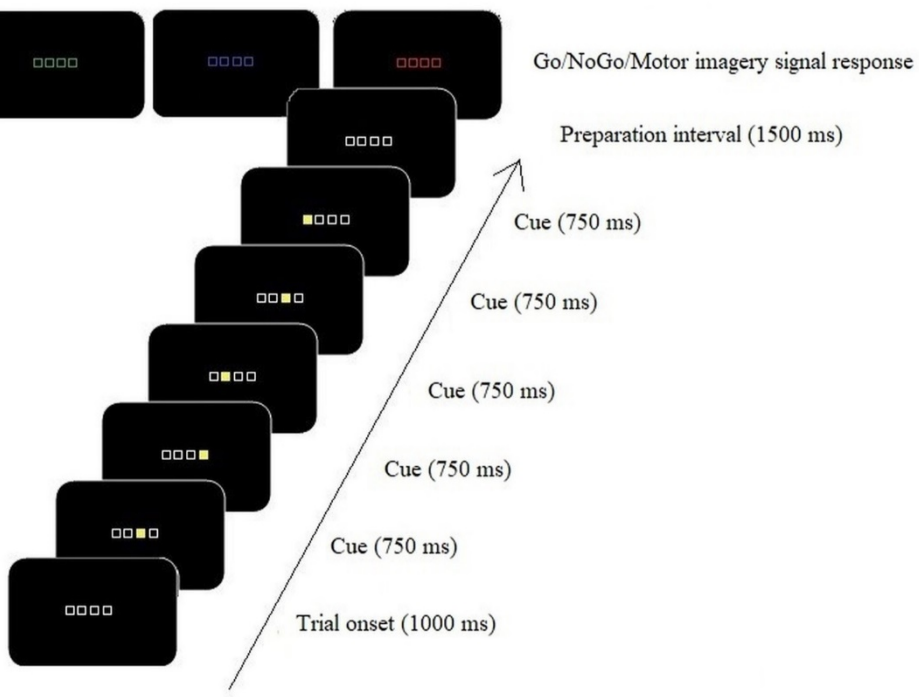
handed, M_{age} = 25.08, SD 3.32; 2) *cathodal* – seven female, five male, 11 right-handed, one left-handed M_{age} = 25, SD 4.47; 3) *sham* – ten female, two male, 11 right-handed, one left-handed M_{age} = 23.08, SD 2.87; 4) *control* – eleven female, one male, 11 of them were right-handed, and one of them was left-handed M_{age} = 24.92, SD 4.03. Participants (except for the control group) were informed about the possibility that they would feel a slight tingling sensation during stimulation. The current study was approved by the local ethics committee of the Adam Mickiewicz University and was performed in accordance with the Declaration of Helsinki.

Stimuli and task

At the start of the experiment, all participants placed their little finger, ring finger, middle finger, and index finger of the non-dominant hand on the *a, s, d, f* keys of a computer QWERTY keyboard. A trial started with a default picture with four horizontally arranged squares presented in the center of the screen. The squares were black with a gray border and were presented on a black background. An overview of the sequence of stimuli is displayed in Figure 1. The four squares spatially corresponded with four response keys (e.g., the left most square corresponded with the "a" key, and the right most square corresponded with the "f" key). Each trial started with a beep of 300 Hz for 300 ms. After 1000 ms five squares, one after another, turned yellow, each for 750 ms (Figure 1). After a preparation interval of 1500 ms relative to stimulus offset, a response cue was presented by changing the color of the borders. In the practice phase, the sequence had to be executed after a green border (Go signal), the sequence had to be mentally imagined after a blue border (Go signal), and after a red border nothing had to be done (NoGo signal), so the action should be inhibited. In the case of a Go signal, participants should either press or imagine pressing the corresponding keys in the same order as in the stimulus sequence. The sequence applied for each condition (i.e., motor

execution, motor imagery, and motor inhibition) was unique and was repeated throughout the practice phase. In the test phase, only a green border was presented, because all sequences had to be physically executed. The employed sequences per condition were the same as in the practice phase, but now all sequences had to be physically executed. Participants were instructed to respond as fast and accurately as possible after presentation of the Go/NoGo signal.

Figure 1. An overview of sequence presentation in the Go/NoGo discrete sequence production (DSP) task. In the practice phase, a Go/NoGo/Motor imagery signal was indicated by three possible informative cues at the end of the stimulus sequence: a green border implied that the sequence had to be executed (Go signal), a blue border indicated that execution of the sequence had to be mentally imagined (Go signal) while a red border indicated that the sequence had to be inhibited.



Procedure

At the start of the experiment, participants received oral instructions about the experiment. They were asked to sit comfortably on a chair at a desk in a dimly lit room. The monitor was placed right in front of them at a distance of 70 cm. Participants were instructed not to move their fingers or contract their muscles during motor imagery and the control condition (i.e., motor inhibition). If participants incorrectly pressed a button during motor execution, information about this error was given. Feedback about incorrect responses ("incorrect response" was displayed) was also given when participants pressed the button before the Go/NoGo signal or when a false button press was made only in the motor execution trials (both in the practice and the test phase). Halfway each block and after each block, participants could relax during a pause. During these pauses, participants were informed about their mean response times (RTs) and percentage of correct responses (PC).

Participants were randomly assigned to one of four groups, i.e., the anodal, cathodal, sham, and control group - 12 participants in each group. Every participant received the same instruction: either to execute a sequence, to imagine a sequence or to do nothing (withhold a response). In the case of motor imagery, participants were instructed to simulate a movement from a first-person perspective, i.e., to imagine the execution of a sequence. They were asked to *feel* a movement. To be certain that participants understood the required task as motor imagery instead of visual imagery, they were given examples for each type of imagery: "imagine as if you are walking – you imagine your movements during walking" (for motor imagery) and "imagine yourself walking on the street – you can see yourself walking" (for visual imagery), (Sobierajewicz J. , Przekoracka-Krawczyk, Jaśkowski, Verwey, & van der Lubbe, 2017). Participants were also told to imagine only the sensation of executing a sequence instead of memorizing numbers, symbols or sounds.

The experiment was divided into a practice phase (40 minutes) and a test phase (30 minutes). The time between the end of the practice phase and the start of the test phase lasted

approximately five minutes. During the practice phase, participants performed two blocks consisting of 96 sequences which had to be executed (32 sequences), imagined (32 sequences), or inhibited (32 sequences). The test phase consisted of one block with 128 sequences which now all had to be executed, including sequences from the practice phase: 32 familiar imagined before, 32 familiar executed before, 32 familiar inhibited before, and 32 unfamiliar new sequences. The different type of sequences were randomized within blocks.

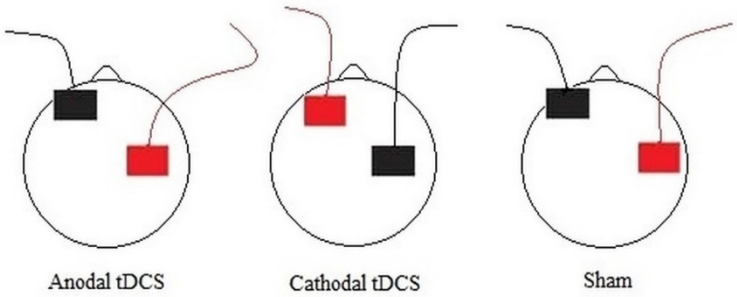
In our experiment, six different structures of movement sequences were created with four response variants (12432, 13423, 14213, 13241, 14312, and 21431). The sequences which were used in the experiment are shown in the Appendix. This procedure enables to eliminate finger-specific effects and to maintain the same level of complexity for all participants. The presented sequences were counterbalanced across participants and fingers.

tDCS

The electrical stimulation was carried out with a battery driven stimulator (BrainSTIM, Italy). tDCS was delivered during the practice phase through two saline-soaked sponge electrodes with a surface area of 35 cm^2. ~~which were impregnated with a saline solution~~. In the case of anodal stimulation, the active electrode was placed over the primary motor cortex contralateral to the non-dominant hand according to the international 10-20 system of electrode placement (C3/4), (Saimpont, et al., 2016; Foerster, et al., 2013; Cuypers, et al., 2013). The reference electrode was located ipsilaterally relative to the non-dominant hand over the supraorbital region (Fp1/Fp2). The electrode positions were exchanged in the cathodal stimulation condition (Figure 2). Thus, regardless of the type of stimulation (anodal, cathodal or sham), for right-handed participants the electrodes were placed on the left supraorbital area and right primary motor area, and for left-handed participants on the right supraorbital area and left primary motor area.

The practice phase lasted 40 minutes, and tDCS started in parallel with the practice phase. The constant direct current ramped up for 30 s until it reached an intensity of 2 mA. It was kept constant for 15 minutes, before ramping down over 30 s to 0 mA. During sham stimulation, the electrode montage was identical to the anodal stimulation. The current also increased over the first 30 s to ramp down in 30 s and was turned off without informing the participant. Sham stimulation is aimed to elicit the same sensation of current onset as in real stimulation (i.e., anodal or cathodal) but should not result in depolarization or hyperpolarization. Apart from the control group, all participants were informed that they could receive either real (anodal/cathodal) or sham stimulation, but they were informed that these three conditions would feel the same (e.g., tingling, itching). We did not test whether participants were able to distinguish between real or sham stimulation.

Figure 2. Example of electrode montage for anodal tDCS (the active electrode was placed over the primary motor cortex contralateral to the non-dominant hand while the reference electrode was located ipsilaterally to the non-dominant hand over the supraorbital region), cathodal tDCS (the electrode positions were exchanged relative to anodal tDCS), and for sham stimulation for a right-handed participant. For the tDCS conditions red represents the anode and black represents the cathode.



EMG

The electromyographic (EMG) activity was recorded during the practice phase to control whether a movement occurred only on those trials that required physical execution (Miyaguchi, et al., 2013; Saimpont, et al., 2016). EMG was measured bipolarly by attaching the electrodes on the musculus flexor digitorum superficalis (which enables to record finger movements) and on the processus styloideus ulnae of the non-dominant hand.

EMG was recorded with Vision Recorder (Brain Products – version 2.0.3). Offline, analyses were performed with Brain Vision Analyzer (version 2.0.4) software. The EMG signal was low-pass filtered at 50 Hz (24 dB/oct) and high-pass filtered at 20 Hz (24 dB/oct). The threshold for a movement was set at 40-90 µV depending on the resting level of the individual participant. A Complex Morlet wavelet was chosen (c=5) to extract the relevant muscle activity, with the lower and upper boundaries for the extracted layer set at 20 and 50 Hz, respectively (Carillo-de-la-Peña, Galdo-Álvarez, & Lastra-Barreira, 2008).

The execution period included 6000 ms starting from the Go/NoGo signal during which the sequence was executed, imagined or inhibited. After a logarithmic transformation, separate repeated measures ANOVAs were carried out with Task (3), (motor execution, motor imagery, motor inhibition trials) and Group (4) as factors, to determine whether participants selectively contracted their muscles only during motor execution in the practice phase.

Response parameters

Response time (RT) was defined as the time interval between the onset of the Go signal and the depression of the first key, and subsequently as the time between two consecutive key presses within a sequence (Ruitenberg, De Kleine, Van der Lubbe, Verwey, & Abrahamse, 2011; De Kleine & Van der Lubbe, 2011). Only RTs from correct responses were analyzed. A

trial was considered incorrect when the button was pressed before the Go/NoGo signal or when a false button (in the wrong order) was pressed. The Percentage Correct (PC) for each block indicated the number of fully correct responses in all Go trials. We divided each practice block into four parts (i.e., subblocks) to examine more precisely the effect on RT and PC during the practice phase between groups. Mean RTs in each practice block were evaluated statistically by an analysis of variance (ANOVA) with repeated measures with Subblock (4), and Key (5) as within-subject factors, and Group (4), (anodal, cathodal, sham, and control group) as between-subjects factor. The test phase involved a repeated measures ANOVA with the factors Type of Sequence (4), (familiar executed, familiar imagined, familiar inhibited and unfamiliar sequences), Key (5), and Group (4). To perform more detailed analyses for keys (to differentiate the initiation time and the execution time), we decided to reduce the number of levels of the variable Key from five to two (including the first key press and the average of keys 2 to 5), (Sobierajewicz, Szarkiewicz, Przekoracka-Krawczyk, Jaśkowski, & van der Lubbe, 2016; Sobierajewicz J. , Przekoracka-Krawczyk, Jaśkowski, Verwey, & van der Lubbe, 2017).

Error analyses were performed on arcsin transformed error proportions to stabilize variances. For the practice phase, repeated measures ANOVAs were performed for each practice block with Subblock (4) as within-subjects factor, and Group (4; anodal, cathodal, sham, and control group) as between-subjects factor. The test phase involved a repeated measures ANOVA with the factors Type of Sequence (4; familiar executed, familiar imagined, familiar inhibited and unfamiliar sequences), and Group (4) as between-subjects factor.

All statistical analyses were performed with STATISTICA 12®. The threshold for significant effects was fixed at $p < .05$. Greenhouse-Geisser epsilon correction was applied to the analyses whenever appropriate. Post-hoc tests involved Tukey`s HSD test. To increase sensitivity for detecting gradual differences as a function of Subblock, we examined linear, quadratic, and cubic contrasts.

Results

The practice phase

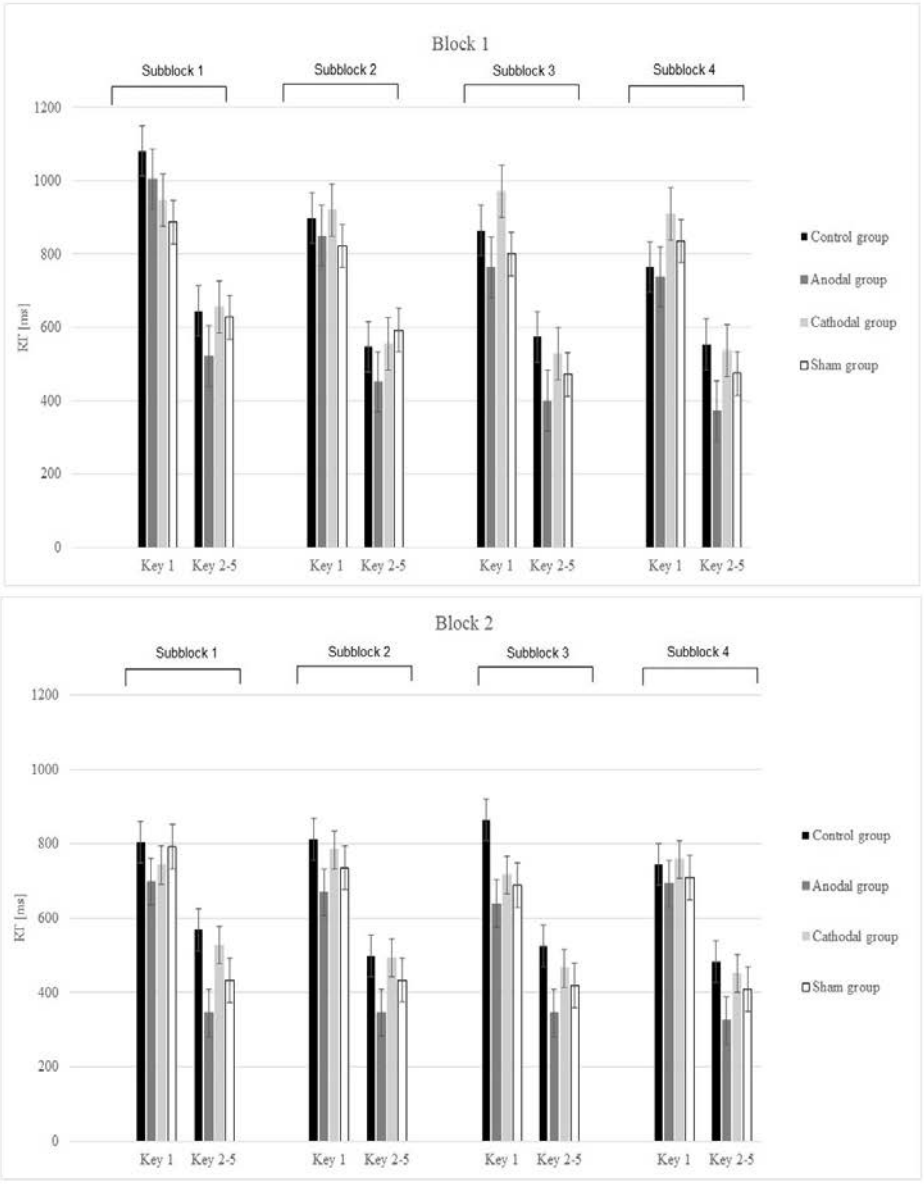
RT.

Figure 3 gives an overview of mean RTs results from the two blocks of the practice phase for each group as a function of Key. The analysis performed for the first block revealed no difference in mean RTs between groups, $F(3, 44) = 1.3$, $p = .29$, $\eta_p^2 = .08$. RTs changed as a function of Subblock $F(3, 132) = 22.49$, $\epsilon = .76$, $p < .001$, $\eta_p^2 = .34$, (linear trend: $F(1, 44) = 36.09$, $p < .001$; quadratic trend: $F(1, 44) = 8.16$, $p = .007$), indicating a general decrease in RT for all groups during the first block of learning a motor skill. No interaction between Subblock and Group was observed, $p = .19$, suggesting that the decrease of RT was similar in all groups. A main effect of Key was observed, $F(1, 44) = 208.46$, $p < .001$, $\eta_p^2 = .83$. Inspection of Figure 3 shows that RT in the first block for the first key was longer than for the subsequent keys. No interaction between Key and Group was observed, $p = .4$. No significant interaction between Subblock and Key was observed, $p = .48$. Importantly, a significant interaction between Subblock, Key, and Group was observed, $F(9, 132) = 3.68$, $p = .001$, $\eta_p^2 = .2$ (linear × linear trend: $F(3, 44) = 7.03$, $p = .001$). To clarify this interaction, separate ANOVAs for each key were performed with Subblock (4) and Group (4) as factors. The analysis for the first key press revealed no significant difference between groups, $F(3, 44) = .76$, $p = .53$, $\eta_p^2 = .05$. The time required for the first key press changed as a function of Subblock, $F(3, 132) = 11.25$, $\epsilon = .8$, $p < .001$, $\eta_p^2 = .2$, (linear trend: $F(1, 44) = 21.13$, $p < .001$), indicating a general decrease of RT in all groups during the first block of learning a motor skill. A significant interaction between Subblock and Group was observed, $p = .02$. Separate analyses per Group revealed that RTs changed as a function of Subblock in the control group: $F(3, 33) = 11.86$, $\epsilon = .52$, $p < .001$, $\eta_p^2 = .52$, and in the anodal group: $F(3, 33) = 10.96$, $\epsilon = .67$, $p < .001$, $\eta_p^2 = .5$. The analysis

performed for the average of keys 2 to 5 revealed no significant difference in RTs between groups, $F(3, 44) = 1.95$, $p = .14$, $\eta_p^2 = .12$. The time needed to execute the rest of the sequence changed as a function of Subblock, $F(3, 132) = 27.43$, $\epsilon = .62$, $p < .001$, $\eta_p^2 = .38$, (linear trend: $F(1, 44) = 36.6$, $p < .001$; quadratic trend: $F(1, 44) = 16.77$, $p < .001$), also indicating a general decrease of RT in all groups during the first block of learning a motor skill. No significant interaction between Subblock and Group was observed, $p = .13$.

The analysis performed for the second block of the practice phase also revealed no significant difference in mean RTs between groups, $F(3, 44) = 2.03$, $p = .12$, $\eta_p^2 = .12$. RTs changed as a function of Subblock, $F(3, 132) = 3.46$, $\epsilon = .7$, $p = .03$, $\eta_p^2 = .07$, (linear trend: $F(1, 44) = 9.54$, $p = .003$), indicating a decrease in RT in all groups in the second practice block. No interaction between Subblock and Group was observed, $p = .31$. A main effect of Key was observed, $F(1, 44) = 243.61$, $p < .001$, $\eta_p^2 = .85$. No interaction between Key and Group was observed, $p = .63$. No significant interaction between Subblock and Key was observed, $p = .78$; and no significant interaction between Subblock, Key and Group was observed, $p = .3$.

Figure 3. Response times (RTs) in milliseconds (ms) from two separate blocks of practice phase for all groups as a function of Key. *Error bars* represent standard errors.

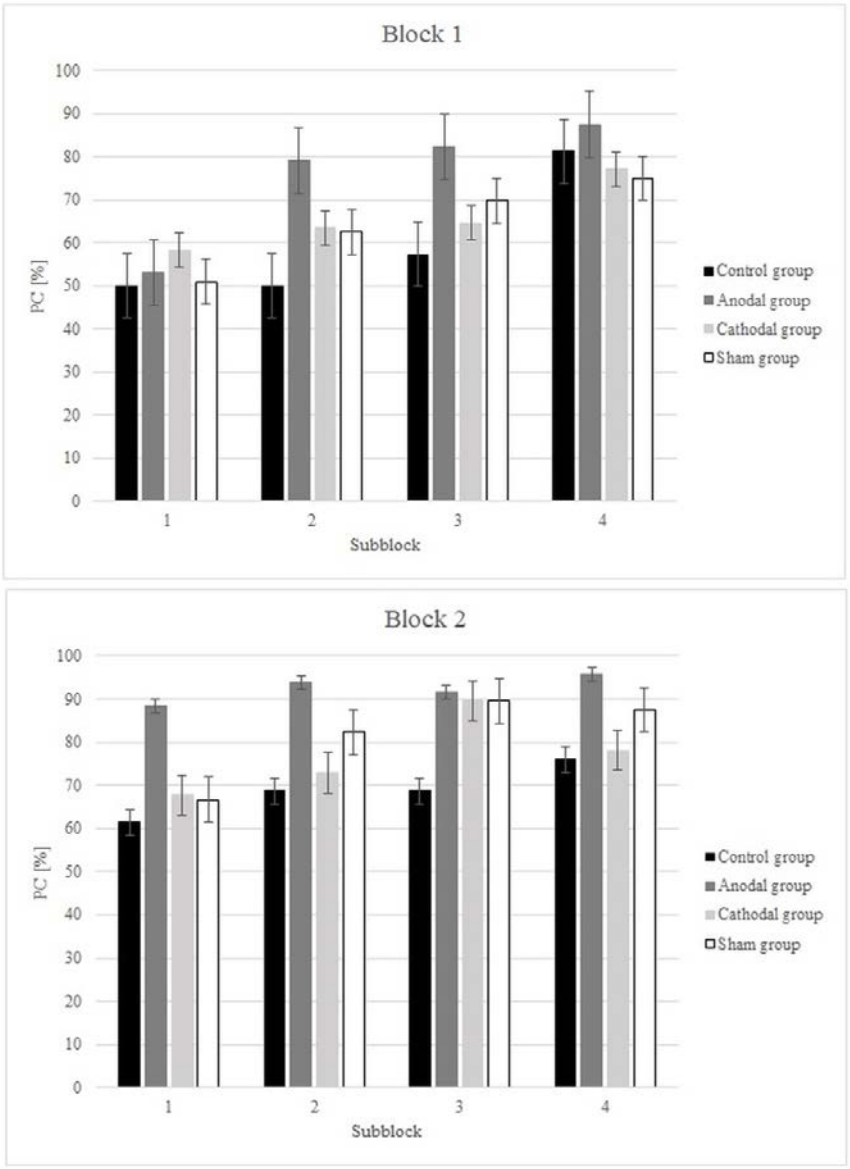


PC.

A repeated measures ANOVA was performed on arcsin transformed error percentages as a function of Group (4) and Subblock (4) for each block of the practice phase. In the first practice block, no significant difference in accuracy was observed between the groups, $F(3, 44) = 2.06$, $p = .12$, $\eta_p^2 = .12$ (Figure 4). A main effect of Subblock was observed, $F(3, 132) = 17.99$, $\epsilon = .89$, $p < .001$, $\eta_p^2 = .29$, (linear trend: $F(1, 44) = 49.55$, $p < .001$), indicating that response accuracy increased with practice. No significant interaction between Subblock and Group was observed, $p = .18$. In the second practice block, a significant difference in accuracy

between groups was observed, $F(3, 44) = 5.87$, $p = .002$, $\eta_p^2 = .29$. Post hoc tests only revealed that participants in the anodal group responded more accurately than participants in the control group, $p = .001$. A main effect of Subblock was observed, $F(3, 132) = 12.11$, $\epsilon = .72$, $p < .001$, $\eta_p^2 = .22$, (linear trend: $F(1, 44) = 31.59$, $p < .001$; quadratic trend: $F(1, 44) = 4.68$, $p < .001$). No significant interaction between Subblock and Group was observed, $p = .11$.

Figure 4. Percentage of correct response (PC) for each Block of the practice phase as a function of Subblock. *Error bars* represent standard errors.

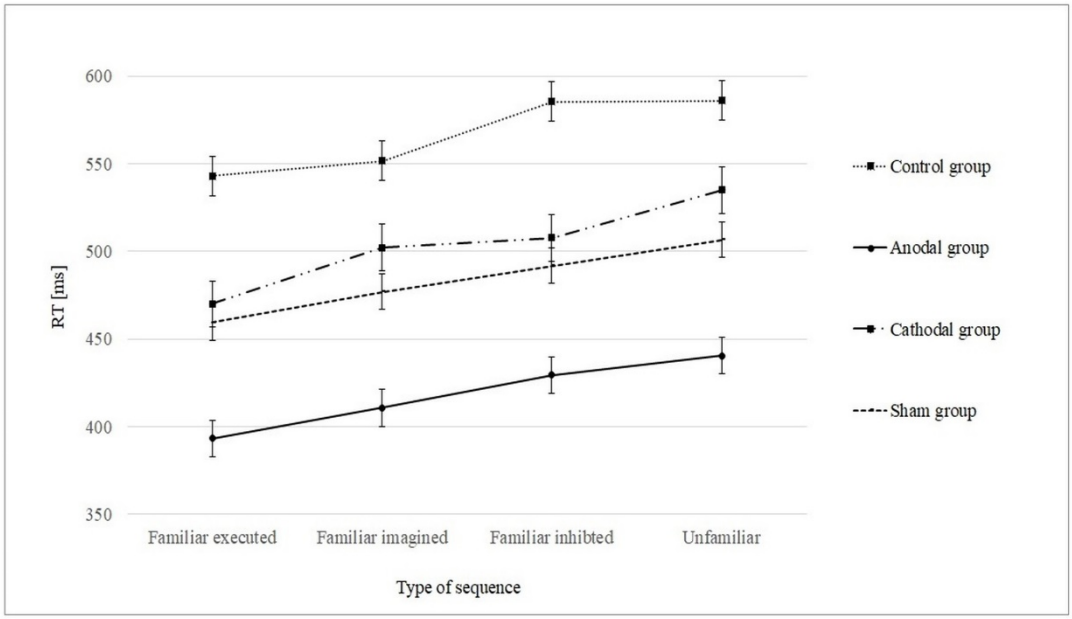


The test phase

RT.

In the test phase, the sequences that were executed, imagined, or inhibited in the practice phase now all had to be executed. Results showed significant differences in mean correct response time between the groups, $F(3, 44) = 3.24$, $p = .03$, $\eta_p^2 = .18$ (Figure 5). Post-hoc tests revealed that participants in the anodal group executed sequences faster than participants in the control group, $p < .02$. No other significant differences between groups were observed, $p > .24$. A significant difference as a function of Type of Sequence was observed, $F(3, 132) = 22.87$, $\epsilon = .83$, $p < .001$, $\eta_p^2 = .34$. Post-hoc test revealed that familiar executed sequences were executed faster than unfamiliar sequences, $p < .001$, and familiar imagined sequences were also executed faster than unfamiliar sequences, $p < .001$. No significant difference was observed between unfamiliar and familiar inhibited sequences, $p = .2$; and no significant difference was observed between familiar imagined sequences and familiar inhibited sequences, $p = .08$. Post-hoc tests also revealed that familiar executed sequences were carried out faster than familiar imagined sequences, $p < .001$, and familiar inhibited sequences, $p < .001$.

Figure 5. Mean response times (RTs) in milliseconds (ms) in the test phase for all groups as a function of Type of Sequence. *Error bars* represent standard errors.



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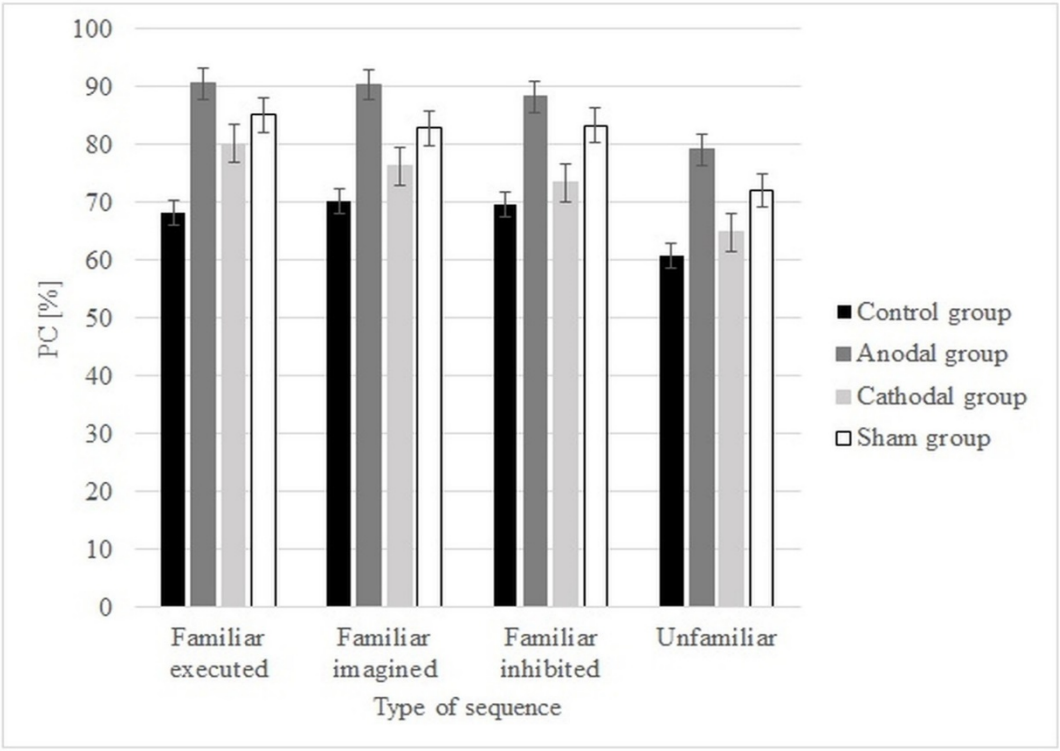
393 No significant interaction between Type of Sequence and Group was observed, $p = .68$. A main
394 effect of Key was observed, $F(4, 176) = 129.53$, $\epsilon = .5$, $p < .001$, $\eta_p^2 = .75$, but no interaction
395 between Key and Group was observed, $p = .74$. An interaction between Type of Sequence and
396 Key was observed, $F(12, 528) = 2.8$, $\epsilon = .59$, $p < .001$, $\eta_p^2 = .06$. Separate *t*-tests for the first
397 key revealed that the time to initiate a sequence was faster for familiar executed sequences as
398 compared with familiar imagined, $t(47) = 2.77$, $p = .008$; familiar inhibited, $t(47) = 4.99$, $p <$
399 .001; and unfamiliar sequences, $t(47) = 4.14$, $p < .001$. The first key press was also faster for
400 familiar imagined sequences than for familiar inhibited sequences, $t(47) = 3.13$, $p = .003$; and
401 unfamiliar sequences, $t(47) = 2.76$, $p = .008$. No significant difference in initiation was observed
402 between familiar inhibited and unfamiliar sequences, $t(47) = .36$, $p = .07$. For the average of 2-
403 5 keys, results revealed faster execution for familiar executed sequences as compared with
404 familiar imagined, $t(47) = 3.7$, $p = .001$; familiar inhibited, $t(47) = 5.02$, $p < .001$; and unfamiliar
405 sequences, $t(47) = 5.25$, $p < .001$. No significant difference in execution was observed between
406 familiar imagined and familiar inhibited sequences, $t(47) = 1.03$, $p = .03$. Results revealed

slower execution in the case of unfamiliar sequences relative to the familiar imagined, $t(47) = 3.19$, $p < .003$; and familiar inhibited sequences, $t(47) = 2.14$, $p < .04$.

PC.

In the test phase, a similar repeated measures ANOVA was performed on arcsin transformed PCs as a function of Group (4), and Type of Sequence (4). A significant difference in accuracy was observed between groups, $F(3, 44) = 3.49$, $p = .02$, $\eta_p^2 = .19$ (Figure 6). Post hoc tests revealed that the anodal group made less errors than the control group, $p = .02$. No other significant differences were observed between groups, $p > .05$. A main effect of Type of Sequence was observed, $F(3, 132) = 9.17$, $\epsilon = .7$, $p < .001$, $\eta_p^2 = .17$. Post hoc tests only revealed that unfamiliar sequences were executed less accurately than familiar executed, familiar imagined, and familiar inhibited sequences, $p < .001$. Inspection of Figure 6 clearly shows that the lowest number of correct responses were observed in all groups in the case of unfamiliar sequences (not practiced before). No significant interaction between Type of Sequence and Group was observed, $p = .98$.

Figure 6. Percentage of correct response (PC) in percentages (%) in the test phase for each group as a function of Type of Sequence. *Error bars* represent standard errors.

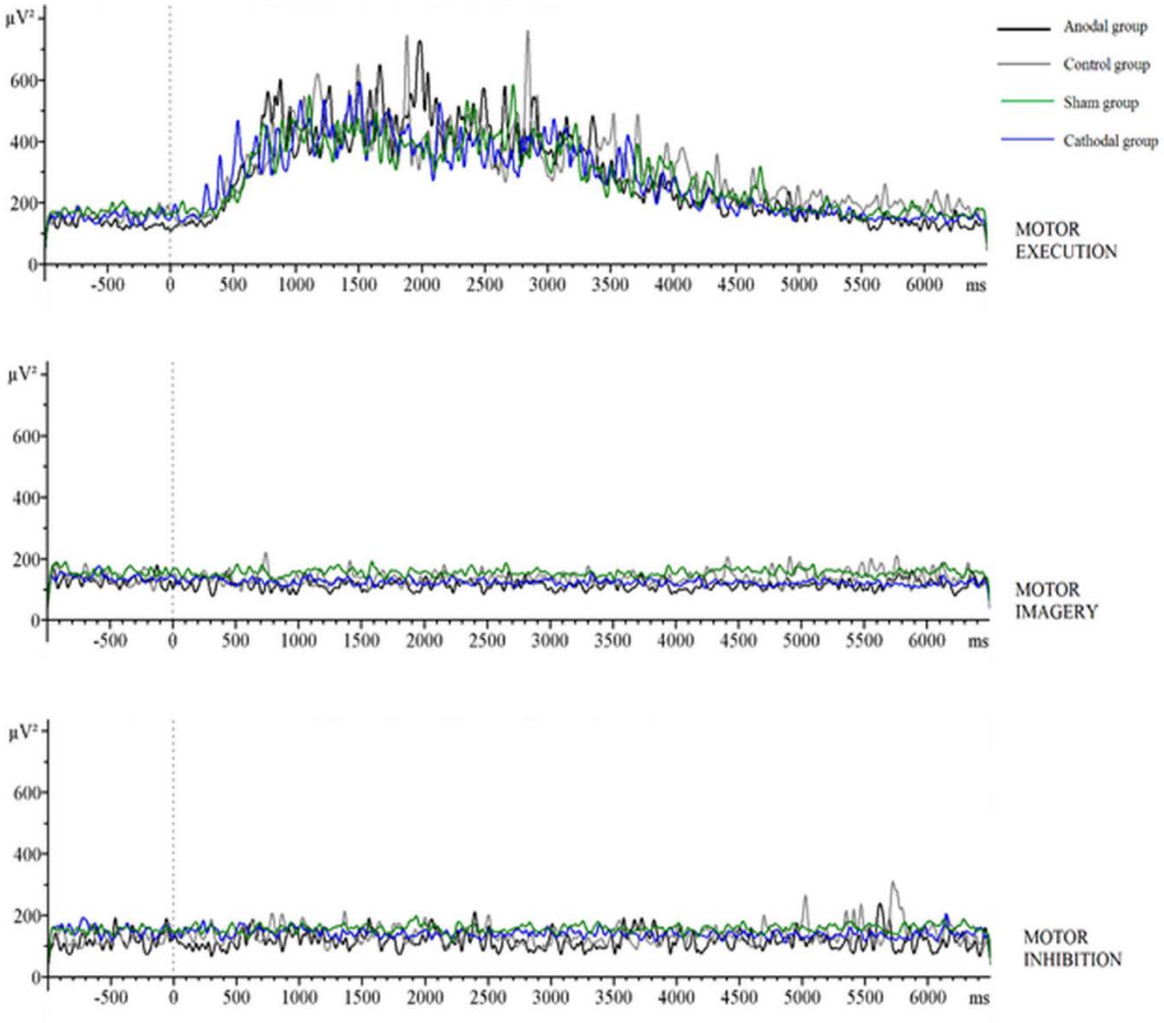


EMG

Figure 7 shows the EMG signal related to the non-dominant hand while performing the required motor task in the practice phase (i.e., during motor execution, motor imagery, and motor inhibition). First, we compared EMG as a function of Task; secondly, we focused on the comparison of motor imagery and motor inhibition to establish whether participant really did not flex their muscles during motor imagery. Figure 7 shows that in all groups the EMG signal was larger for executed sequences than for imagined and inhibited sequences. The EMG signal did not differ between groups, $F(3, 42) = 1.12$, $p = .35$. A significant difference was observed as a function of Task, $F(2, 84) = 123.25$, $\epsilon = .56$, $p < .001$, $\eta_p^2 = .75$. Separate *t*-tests revealed that the EMG signal during motor execution was larger than during motor imagery, $t(47) = 7.6$, $p < .001$; the EMG signal was also larger in the case of motor execution as compared with motor inhibition, $t(47) = 7.8$, $p < .001$. We were especially interested whether there was a significant

difference between motor imagery and motor inhibition. The reason is because in the case of motor imagery participants were asked only to imagine performing a motor sequence, as a consequence they could unintentionally induce some muscles tension. Results revealed no difference in EMG activity during motor imagery and motor inhibition, $t(45) = 1.75$, $p = .09$. In conclusion, the results of our EMG analyses revealed that participants in all groups moved their fingers mainly in the case of motor execution and they did not flex their muscles during motor imagery and motor inhibition.

Figure 7. Outcome of the wavelet analysis performed on the raw EMG signal measured from the electrodes attached to the non-dominant hand in the practice phase. The grand averages are presented for all groups, for motor execution, motor imagery and motor inhibition -1000 ms before the Go/NoGo signal (0 ms) to 6000 ms.



Discussion

In the present study, we wanted to establish the influence of tDCS on learning a fine sequential hand motor skill by examining both non-specific learning effects and sequence-specific learning effects. Furthermore, we wanted to investigate whether learning by motor imagery combined with tDCS might increase sequence-specific learning effects. In order to examine this, we stimulated the primary motor cortex of the non-dominant hand to be used in the Go/NoGo DSP task, which allows to estimate both non-specific and sequence-specific learning effects. As an extension relative to previous studies (Cuypers, et al., 2013; Foerster, et al., 2013; Saimpont, et al., 2016), we involved four groups of participants: an anodal, cathodal, sham stimulation group, and a control group (without stimulation). First, we will concentrate on non-specific learning effects of tDCS by comparing learning effects between groups. Secondly, we will focus on sequence-specific learning effects of tDCS on motor learning by comparing familiar sequences with unfamiliar sequences between groups. Finally, we will answer the question whether learning a fine motor skill with motor imagery is boosted by tDCS.

First, we questioned to what extent tDCS affects the learning of a fine motor skill. In the final test phase a significant difference in mean RTs was observed only between the anodal group and the control group, i.e., participants in the anodal group executed sequences significantly faster than the control group. Comparable effects were observed for accuracy, i.e., results showed that participants in the anodal group made less errors relative to the control group. Besides the fact that all groups were faster and more accurate with practice (indicating non-specific learning effects), we revealed that tDCS increased motor performance only when comparing the results of the anodal group with the control group. In other words, our results showed that anodal tDCS affects non-specific learning effects, in line with previous studies (Cuypers, et al., 2013; Ciechanski & Kirton, 2016). These results are also consistent with findings reported by Antal et al. (2004). They observed improved motor performance on a

visuo-motor task after anodal, but not after cathodal stimulation, although this was only observed in the initial learning phase. Antal et al. (2004) explained these findings by the improvement of perceptual-motor performance, which can be related with improved visual perception or cognitive processing (see: (Antal, Nitsche, Kruse, Hoffmann, & Paulus, 2004). In our study, more long-lasting effects of anodal stimulation were observed, as group differences were already visible in the practice phase, and remained present in the test phase, which may be due to different task procedure. Nevertheless, our results also indicate that anodal stimulation affects non-specific learning effects which can be explained by the increased familiarity with the task procedure instead of learning of a particular motor sequence (Sobierajewicz J. , Przekoracka-Krawczyk, Jaśkowski, Verwey, & van der Lubbe, 2017). Our results are also consistent with the findings of earlier studies that showed increased learning effects with anodal tDCS (Nitsche, et al., 2003; Reis, et al., 2009; Stagg, et al., 2011), but only when anodal tDCS was applied *during* the required motor task. Stagg et al. (2011) demonstrated that the effects of tDCS are time-dependent, i.e. anodal tDCS applied during the task increased motor learning while either anodal or cathodal tDCS applied *before* the motor task diminished learning (as compared with sham stimulation). Based on results of Stagg et al. (2011) it may be hypothesized that application of tDCS before the practice phase in our experiment could have led to diminished learning. Thus, the moment of applying tDCS seems to have a crucial role in learning effects of brain stimulation, this seems also quite relevant for neurorehabilitation practices that aim to help patients with for example stroke to recover their motor functions.

In the current study, we also included a control group, which may allow to examine whether tDCS effects are possibly due to a placebo effect. Similar results for the sham stimulation and the control group would indicate that a placebo effect for sham stimulation is unlikely. Although no difference between sham stimulation and the control group was observed, we also did not observe any difference in motor performance (i.e., motor execution of a

sequence) between sham stimulation, anodal stimulation, and cathodal stimulation. Our results revealed that anodal stimulation leads to a significant improvement of motor performance but only when compared with the control group. In contrast to previous studies (Kang & Paik, 2011; Cuypers, et al., 2013; Kidgell, Goodwill, Frazer, & Daly, 2013), we did not observe a significant difference between the anodal group and the sham group. These results indicate that we cannot exclude the possibility that participants improved performance because they were stimulated (either real or sham stimulation) which may reflect a placebo effect (Aslaksen, Vasylenko, & Fagerlund, 2014). For example, it might be the case that as participants were stimulated they became more motivated to carry out the task. In order to examine the potential benefit of tDCS due to a modulation of local excitability, one could use EEG and/or transcranial magnetic stimulation (TMS). For example, anodal tDCS above the primary motor cortex is thought to increase cortical excitability which can be observed by an increase in the hand motor evoked potential (MEP), while cathodal stimulation leads to a decrease in MEP amplitude (Nuzum, Hendy, Russell, & Teo, 2016). Another method to measure the effects of tDCS is EEG, which allows to observe the influence on spectral power and event-related desynchronization (ERD) due to tDCS (Mondini, Mangia, & Cappello, 2018). The examination of MEP or ERD can be useful to determine the efficacy of tDCS above the primary motor cortex.

Our second aim of this study refer to the influence of tDCS on sequence-specific learning, which was examined by comparing familiar sequences with unfamiliar sequences between groups. Results confirmed our previous findings that familiar sequences were executed more efficiently than unfamiliar sequences (Sobierajewicz, Szarkiewicz, Przekoracka-Krawczyk, Jaśkowski, & van der Lubbe, 2016; Sobierajewicz J. , Przekoracka-Krawczyk, Jaśkowski, Verwey, & van der Lubbe, 2017). However, we did not observe any influence of tDCS on sequence-specific learning effects as similar effects were observed in all groups (i.e., anodal group, cathodal group, sham group, and control group). It can be argued that the number

of participants was not enough to demonstrate the effects of stimulation on sequence-learning. Thus, a potential limitation of this study may arise from the fact that the statistical power was too low. Nevertheless, our results showed the influence of tDCS on non-specific learning effects. Therefore, we think that tDCS has an influence on non-specific learning but not on sequence-specific learning.

The third aim of this study was to examine and better understand the effects of the combination of motor imagery with tDCS. We were interested whether learning a fine motor skill with motor imagery may further increase due to tDCS. Although results showed that anodal tDCS improved motor performance, we revealed that it does not increase the influence of motor imagery on sequence learning, neither in terms of speed nor accuracy. As mentioned-above, we observed that motor imagery itself influenced sequence-specific learning, but this effect was present in all groups. These results indicate that tDCS did not reinforce the effect of motor imagery on learning a sequential motor skill. For a comparison with studies of Saimpont et al. (2016) and Foerster et al. (2013), only non-specific learning effects will be discussed as sequence-specific learning effects were not examined in these studies. In contrast to the study of Saimpont et al. (2016), our results did not reveal that tDCS combined with motor imagery improved the accuracy of motor responses. In their study, the improvement of accuracy has been observed after anodal stimulation combined with motor imagery relative to motor imagery combined with sham stimulation and tDCS alone. On the other hand, Foerster et al. (2013) revealed the improvement of motor performance after anodal tDCS combined with motor imagery, but no effect of training with motor imagery alone was observed. It should be underlined that in our study and in the study of Foerster et al. (2013) and Saimpont et al. (2016) the position of the electrodes, execution of the required task only with the non-dominant hand and the intensity of the current were the same (only the duration of tDCS in our study lasted 15 minutes, while in their studies stimulation lasted 13 minutes). Therefore, results from the above-

553 mentioned studies and our study suggest that the effect of tDCS combined with motor imagery
554 depends more on the amount and quality of motor imagery rather than the duration or intensity
555 of brain stimulation. It should also be noted that individual neuroanatomy might have relevant
556 role in determining the behavioral effects of stimulation. Variability in the efficacy of tDCS
557 may be caused by a variation in electrically generated fields, which can depend on both
558 experimental parameters (e.g., intensity of the current, stimulation duration, etc.) and individual
559 anatomic features of the head and the brain. In the study of Rich et al. (2017), it was revealed
560 that individual variability in brain somatic organization may influence surface scalp
561 localization. In particular, reorganization of the primary motor cortex may occur due to
562 neurologic injury, e.g., after stroke, (Rich, et al., 2017). However, in our study only healthy
563 subjects (without any neurological diseases) were examined, therefore the 10/20 EEG
564 coordinate system (based on the anatomical relationship of skull dimensions to underlying brain
565 anatomy) used in our experiment seems justified. Nevertheless, when examining patients after
566 an injury like a stroke, one should be more cautious in determining the proper stimulation area.
567 In our analyses, we could also observe the role of motor preparation during learning a
568 motor skill. For sequences that were inhibited in the practice phase, we observed that
569 participants in the test phase became as accurate but not as fast relative to familiar executed
570 sequences. This can be explained by the presence of motor preparation before the NoGo signal,
571 which enables to mentally practice a sequence. Based on the results from the current study, it
572 may be argued that mere motor preparation improves accuracy, but does not affect the speed of
573 motor performance. This result partially corresponds with our previous study (Sobierajewicz,
574 Szarkiewicz, Przekoracka-Krawczyk, Jaśkowski, & van der Lubbe, 2016), in which we showed
575 that motor preparation may be sufficient to acquire a motor skill. In this study, we showed that
576 the requirement to imagine a motor sequence was not necessary to demonstrate a learning effect
577 (see: (Sobierajewicz, Szarkiewicz, Przekoracka-Krawczyk, Jaśkowski, & van der Lubbe,

2016)). Hence, it can be concluded that not only motor imagery, but also motor preparation may be beneficial for the learning of a fine motor skill (especially with regard to its accuracy).

In summary, this study showed that anodal tDCS improved both the speed and the accuracy of a motor sequence relative to a control group that received no stimulation. Importantly, tDCS did not facilitate the influence of motor imagery on sequence learning. In other words, tDCS did not boost motor performance after motor imagery training. Future studies are needed to clarify the mixed findings of tDCS, for example by determining the underlying mechanisms with the help of EEG.

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Appendix

Sequences of five key presses used in the experiment:

6 structures of the sequence, 4 versions each
1-**a**, 2-**s**, 3-**d**, 4-**f**
1-**;**, 2-**l**, 3-**k**, 4-**j**

Structure 1

Version_1: Left hand: **a s f d s** (12432) Right hand: **; l j k l** (12432)

Version_2: Left hand: **s d a f d** (23143 Right hand: (23143)

Version_3: Left hand: **d f s a f** (34214) Right hand: (34214)

Version_4: Left hand: **f a d s a** (41321) Right hand: (41321)

Structure 2

Version_1: Left hand: **a d f s d** (13423) Right hand: (13423)
Version_2: Left hand: (24134) Right hand: (24134)
Version_3: Left hand: (31241) Right hand: (31241)
Version_4: Left hand: (42312) Right hand: (42312)

Structure 3

Version_1: Left hand: **a f s a d** (14213) Right hand: (14213)
Version_2: Left hand: (21324) Right hand: (21324)
Version_3: Left hand: (32431) Right hand: (32431)
Version_4: Left hand: (43142 Right hand: (43142)

Structure 4

Version_1: Left hand: **a d s f a** (13241) Right hand: (13241)
Version_2: Left hand: (24312) Right hand: (24312)
Version_3: Left hand: (31423) Right hand: (31423)
Version_4: Left hand: (42134) Right hand: (42134)

Structure 5

Version_1: Left hand: **a f d a s** (14312) Right hand: (14312)
Version_2: Left hand: (21423) Right hand: (21423)
Version_3: Left hand: (32134) Right hand: (32134)
Version_4: Left hand: (43241) Right hand: (43241)

Structure 6

Version_1: Left hand: **a f d a s** (21431) Right hand: (21431)
Version_2: Left hand: (32142) Right hand: (32142)
Version_3: Left hand: (43213) Right hand: (43213)
Version_4: Left hand: (14324) Right hand: (14324)