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Title: Anodal transcranial direct current stimulation over premotor cortex facilitates observational learning of a motor sequence.

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Running title: tDCS facilitates observational learning

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Abstract

Motor skills, including complex movement sequences, can be acquired by observing a model without physical practice of the skill, a phenomenon known as observational learning. Observational learning of motor skills engages the same memory substrate as physical practice, and is thought to be mediated by the action observation network, a bilateral fronto-parietal circuit with mirror-like properties. We examined the effects of anodal tDCS over premotor cortex, a key node of the action observation network, with on observational learning of a serial response time task. Results showed that anodal tDCS during observation of the to-be-learned sequence facilitated reaction times in the subsequent behavioral test. The study provides evidence that increasing excitability of the AON during observation can facilitate later motor skill acquisition.

Introduction

Increasing the excitability of primary motor cortex (M1) with anodal transcranial direct current stimulation (tDCS) has been shown to improve motor performance and increase the acquisition and retention of motor skills (reviews by Reis & Fritsch, 2011; Tanaka *et al*., 2011). Positive effects of anodal tDCS over M1 have been reported for a range of motor skills, from elementary movements (Galea & Celnik, 2009) to slowly acquired complex movement sequences (Reis *et al*., 2009); both explicitly and implicitly learned movement sequences are acquired more quickly with anodal tDCS over M1 during training than with sham stimulation (explicit learning: Reis *et al*., 2009; Marquez *et al*., 2013; Stagg *et al*., 2011; implicit learning: Nitsche *et al*., 2003: Kantak *et al*., 2012). Motor skills, including complex movement sequences, can be acquired by observing a model without physical practice of the skill (Heyes & Foster, 2002; Bird *et al*., 2005). This phenomenon, known as observational learning, occurs for both explicitly and implicitly acquired motor skills, and can produce behavioral change of the same magnitude as physical practice (Heyes & Foster, 2002). Neuroimaging has identified an action observation network (AON), a bilateral fronto-parietal circuit activated by both observation and imitation of actions and so has, in this sense, mirror-like properties (Buccino *et al*., 2004; Cross *et al*., 2009; Roberts *et al*., 2014). Meta-analyses of neuroimaging studies have shown that action observation reliably activates several core nodes in the AON, the inferior and superior parietal lobules, the inferior frontal gyrus, and the adjacent premotor (PM) cortex (Caspers *et al*., 2010; Molenberghs, *et al*., 2012). A division of PM in humans and other primates into functionally distinct dorsal and ventral regions is widely accepted (see reviews by Rizzolatti *et al*., 2002, and Chouinard & Paus, 2006), and there is growing evidence that the ventral division is particularly activated by observation of movement by others and so forms an important node in the AON (see review by Rizzolatti & Craighero, 2004).

Disrupting neural activity in PM (but not frontal cortex) by repeated single transcranial magnetic stimulation (TMS) pulses during action observation reduced the contribution of observational learning to later physical performance (Cantarero *et al*., 2011), indicating an important function of PM in mediating observational motor learning. The purpose of the current study was to determine whether increasing the excitability of PM by applying anodal tDCS during observation of a movement sequence would facilitate observational learning of the sequence.

Materials and methods

Subjects

Forty healthy adults (23 female; median age = 26 years, age range = 18-56 years), with no psychiatric or neurological history and no contraindication to tDCS, took part in the study. Two participated for course requirements and all others were recruited via word-of-mouth. All subjects wrote with their right and were naïve to the purpose of the experiment. The procedures conformed to the Declaration of Helsinki and were approved by the Human Research Ethics Committee of the University of Western Australia. All subjects gave written informed consent and were fully debriefed after task completion. One subject who received sham stimulation reported a mild headache at the end of the procedure.

Apparatus and procedure

The effects of tDCS given during an observational-learning phase were assessed in a subsequent performance-testing phase. During the observation phase, subjects were given either anodal tDCS or sham stimulation while viewing a video of a human right hand pressing computer keys sequentially. The video showed either repetitions of the 12-item key-press sequence used in the subsequent serial reaction time task (SRTT) (the ‘ordered’ condition) or an irregular sequence of key presses (the ‘irregular’ condition). The performance-testing phase, in which subjects did the SRTT, began immediately after viewing one of the videos; no other experimental tasks were imposed. Subjects were randomly assigned to one of the four independent groups formed by the factorial combination of stimulation condition (anodal tDCS, sham) and observation condition (ordered sequence, irregular sequence) with the constraint of equal numbers in each group. For convenience, the four groups are denoted as tDCS/ordered, tDCS/irregular, sham/ordered, and sham/irregular. Subjects were not made aware of the different conditions until after they had finished the experiment.

Observational videos

Each video was composed of a two-minute clip presented four times with two-minute intervals between each presentation, during which the screen was blank. The two-minute clip displayed a model's right hand placed on a computer keyboard, viewed from a similar angle to that of the subject's line of sight of his or her own hand while typing. In the ordered video sequence, the model depressed four keys (V, B, N, or M) in a repeating 12-key sequence identical to that in the SRTT; in the irregular video sequence, these four keys were pressed in an irregular order. The irregular sequence was the same in each block. The model practiced until skilled in both speed and accuracy: no errors were made in the ordered sequence and the mean time between key presses was 330 ms (SD = 5.4) for both videos. An observation period of about six minutes is sufficient to enhance subsequent SRTT performance (Osman *et al*., 2005). Anodal tDCS was administered for 14 minutes, a duration sufficient to affect motor performance (Bolognini, *et al*., 2009; Nitsche & Paulus, 2001). If subjects were to observe the sequence performed continuously for 14 minutes, however, it would be possible for the sequence to be learned completely during the observation period, which would diminish the sensitivity of the subsequent SRTT measure of the amount and the rate of observational learning. To avoid this, two-minute blank screens were positioned between each of the sequence repetitions in the observation videos. This allowed the observation phase to be of sufficient duration for tDCS to be administered for the required 14 minutes, while providing only eight minutes of observation of the sequence repetition.

Transcranial direct current stimulation

Stimulation was delivered by a battery-driven direct-current stimulator (Dupel Iontophoresis System) through a pair of electrodes (4 cm × 6 cm) encased in saline-soaked synthetic sponges, which were secured on each subject's scalp with elastic bandaging. The anode was placed 2 cm anterior and 2 cm medial from C3 in the International EEG 10-20 system to overlie the left premotor cortex (Nitsche, *et al*., 2003). Following standard procedures (Fregni *et al*., 2005), the reference electrode was placed over the right supraorbital region. For anodal stimulation, current was ramped up over ~30 s, held constant at 1 mA for the duration of the observation phase (14 min) and then ramped off over ~30 s. For sham administration, current was ramped up to 1 mA and then immediately ramped off. This method of administration reliably blinds subjects to stimulation condition (Been *et al*., 2007; Fregni *et al*., 2005; Gandiga *et al*., 2006).

Serial reaction time task

The SRTT began immediately after the observation phase. Subjects remained seated in front of the computer screen which displayed four white boxes (2.2 cm × 1.2 cm) separated horizontally by 1 cm against a grey background. Each of the four boxes, from left to right, corresponded to a response key (V, B, N, and M respectively) on a standard keyboard. Subjects were instructed to press these keys with the index, middle, ring, and little finger of their right hand respectively. An asterisk (in 32 point Arial font) appeared in the centre of one of the boxes during each trial. Subjects were instructed to press the corresponding response key as quickly and accurately as possible. The asterisk disappeared immediately a key was pressed, and the next trial began after a 200-ms delay. The asterisks appeared in a repeating 12-key sequence (MBNVBMVNVNMB), identical to the modelled sequence of responses in the ordered-sequence video. The sequence was repeated 60 times, for a total of 720 trials. Accuracy and RT (in ms) were recorded for each trial.

Data analysis

RTs were measured as the time (in ms) between onset of the asterisk and the subsequent response and were initially analysed in 12 blocks of 60 key presses. No negative RTs (where the response preceded the stimulus) were recorded, and only correct responses were included in the calculation of RT. RTs that were more than three standard deviations above an individual's mean in any block were excluded from analysis. No minimum RT boundary was set since very fast RTs can result from anticipating the next stimulus location as the sequence is learned. One subject whose accuracy in one block was below 50% and whose mean accuracy over all blocks was more than 3 SD below the mean was excluded from analysis. Because the independent-group design does not allow comparison of absolute RT measures, RTs were normalized by dividing each subject's median RT in each trial block by their median RT in their first trial block. This normalization allows for comparison of groups while controlling for pre-existing differences in RT and is common practice in the literature (Nitsche, *et al*., 2003; Vogt, 1995). Group means of normalized RTs are reported.

Results

Mean accuracy for each block of 60 trials for each of the four groups is shown in Figure 1. Accuracy decreased with practice (linear effect of Trial Block: $F_{1, 35} = 38.48$, $p < .001$; $\eta_p^2 = .52$). The decrease was small, with an overall decline from 98% to 95% correct, with a decline of no more than 4% in any group. The tDCS groups were slightly more accurate overall than the sham groups (98% and 96% correct respectively; $F_{1, 35} = 5.72$, $p = .022$; $\eta_p^2 = .14$); this small difference in accuracy emerged with block (linear Stimulation by Trial Block interaction: $F_{1, 35} = 5.14$, $p = .03$; $\eta_p^2 = .13$). Mean normalized RTs for each trial block for each group are shown in Figure 2. RTs in all groups decreased with practice (overall effect of

Trial Block: $F_{3.8,\ 134.9} = 17.20$, $p < .001$, $\eta_p^2 = .33$) with significant linear and quadratic components ($F_{1,\ 35} = 34.66$, $p < .001$, $\eta_p^2 = .50$ and $F_{1,\ 35} = 6.96$, $p = .012$, $\eta_p^2 = .17$ respectively). Analysis of all trial blocks showed that the main effect of Observed Sequence (Ordered, Irregular) was not significant ($F_{1,35} = 1.74$, $p = .195$, $\eta_p^2 = .05$) and that the main effect of Stimulation (tDCS, Sham) was significant ($F_{1,35} = 6.84$, $p = .013$, $\eta_p^2 = .16$), reflecting an overall RT advantage in the groups that had received tDCS during observation. This main effect was qualified by a Stimulation x Observed Sequence interaction ($F_{1,\ 35} = 8.32$, $p = .007$; $\eta_p^2 = .19$), reflecting the faster normalized RT in the group that received anodal tDCS while observing the ordered sequence than in the remaining groups. Normalized RTs were similar in the other three groups throughout physical practice. The RT advantage in the group that was stimulated while observing the ordered sequence developed progressively with physical practice, shown in a significant overall three-way interaction of Stimulation, Observed Sequence, and Trial Block ($F_{3.8,\ 134.9} = 2.63$, $p = .039$; $\eta_p^2 = .07$) with a significant linear component ($F_{1,\ 35} = 5.48$, $p = .025$; $\eta_p^2 = .14$). Averaging normalized RTs in blocks of 60 trials for display in Figure 2 obscures the effects that stimulation and order of the observed sequence might have had in the first 60 trials of physical practice. Figure 3 shows the normalized RTs of each group in the first 60 trials in blocks of 12 trials. RTs decreased over these early trial blocks ($F_{3,\ 105} = 7.68$, $p < .001$; $\eta_p^2 = .18$; linear component: $F_{1,\ 35} = 20.83$, $p < .001$; $\eta_p^2 = .37$). Although somewhat faster mean normalized RTs emerged in the first 60 trials in the group that received tDCS while observing the ordered sequence of key presses, neither the main effect of stimulation nor any interaction with stimulation approached statistical significance. Importantly, however, there was an effect of Observed Sequence ($F_{1,\ 35} = 6.82$, $p = .013$; $\eta_p^2 = .16$), with faster mean normalized RTs in the groups that observed an ordered sequence than those that observed an irregular sequence (*M* = .82, *SEM* = .04

and *M* = .94, *SEM* = .03 respectively). Comparing the data in Figure 3 with those in Figure 2 indicates first, that the RT advantage of the group stimulated while observing the ordered sequence developed progressively with physical practice, and second, that the aggregated RT advantage for the two groups that observed the ordered sequence was present in the first block of 60 trials but not in later blocks.

Discussion

The results show that observing an ordered sequence of key presses without acting facilitated RT in the early stages of an immediately subsequent serial reaction time task, indicating that observational learning took place. The RT benefit of having observed the ordered sequence was transitory in the sham/ordered group, and did not persist throughout the prolonged physical practice phase of the SRTT. In contrast, the RT advantage of the tDCS/ordered group increased progressively throughout physical practice of the subsequent SRTT while maintaining accuracy relative to the other groups. This latter finding is the first demonstration that increasing the excitability of an area of the action observation network with anodal tDCS during action observation enhances observational motor learning, and complements the report that observational learning is impaired by disrupting PM activity during observation (Cantarero *et al.*, 2011). Together, these findings indicate that PM is a critical part of a neural circuit that translates movement observation into a motor memory which is then elaborated by physical practice.

The finding of a cumulative facilitation of RT during physical practice in the tDCS/ordered group is of particular interest as it indicates that increasing the excitability of PM during motor observation potentiated the neuroplastic changes induced by subsequent physical practice of the SRTT. This implies a synergy of observational and active motor learning, such that prior observation increases the plasticity of the motor system, making it more responsive to physical practice. The similarity of the behavioral outcomes of motor skill learning by observation and by physical practice has led to the suggestion that observational learning results from activation of the same neural circuits as those engaged by physical practice (Bird *et al*., 2005; Mattar & Gribble, 2005; Roberts *et al*. 2014). Observation of isolated thumb movements in the direction opposite to that evoked by single TMS pulses over M1 shifts the direction of the TMS-evoked movements to the observed direction (Stefan *et al*., 2005); in a subsequent experiment, it was shown that combining observation of thumb movements in the same direction as the physically practiced movements with physical practice led to better learning, shown by a greater probability of a directional shift than physical practice alone (Stefan *et al*., 2008). The authors hypothesized that activation of neurons with mirror-like properties in PM during movement observation contributes to the formation of specific motor memories within M1, a view consistent with the known anatomical and physiological connections between the two areas. Hand representations in the PM have profuse anatomical interconnections with the ipsilateral M1 in monkeys (Dum & Strick, 2005) and functional connections between these areas in humans have been shown by the rapid effects of conditioning TMS pulses over the PM on the amplitude of motor evoked potentials elicited by TMS pulses over the ipsilateral M1 (Civardi *et al*., 2001; Koch *et al*., 2007). Furthermore, anodal tDCS over PM has been shown to induce lasting changes in intracortical inhibitory and facilitatory processes in M1, which the authors attributed to functional connectivity between the two areas and not passive spread of current from the anode positioned over PM (Boros *et al*., 2008).

The hypothesis that PM mediates the translation of action observation to a specific motor memory in M1 is supported by two relevant observations. First, observational motor learning is decreased by disrupting M1 with repetitive TMS *after* observing a model reaching in a novel force field, suggesting that M1 is engaged subsequent to observation in forming a motor memory based on movement observation (Brown *et al*., 2009). Second, observing movements has been shown to potentiate subsequent M1 plasticity induced by paired associative stimulation targeting a hand muscle engaged by the movements seen during the observation phase (Sale & Mattingley, 2013). Together, these observations suggest a confluence of observational and active motor learning, with observation priming plastic change in M1.

Although the finding that anodal DCS during observation of the ordered movement sequence facilitated subsequent physical performance is clear, questions of the temporal and spatial location of the effect deserve consideration. Although stimulation was confined to the observation phase, the changes in neural excitability induced by stimulation parameters comparable to those used in this experiment are known to persist for at least 50 min (Lang *et al*., 2005; Nitsche & Paulus, 2001). Taking this into account, RT facilitation in the tDCS/ordered group could have been due to increased excitability that outlasted stimulation and was present during the physical practice phase. However, the tDCS/irregular group performed no better than the sham/irregular group, showing no beneficial effect of the persisting excitability changes. It might be conjectured that the absence of a beneficial effect of stimulation in the tDCS/irregular group was offset by anterograde interference resulting from observation of the irregular sequence while receiving tDCS. This possibility can be discounted, as there was no evidence of anterograde interference in the two sham groups: indeed, the sham/irregular group performed slightly better than the sham/ordered group.

Although current density in the cortex is greatest immediately under the stimulating electrodes (Miranda *et al*., 2006), it is clear that the standard electrode configuration used in this experiment would have induced widespread changes in cortical and subcortical excitability, making spatial localization of the effect problematic (Lang *et al*., 2005). The effect of applying anodal tDCS over the PM on observational learning may have been mediated, at least in part, by other brain regions. It is possible that the effects of tDCS over PM were mediated by a stimulation-evoked increase in M1 excitability, which has been shown to contain neurons with mirror-like properties in non-human primates (Dushanova & Donogue, 2010; Tkach *et al*., 2007) and so may be engaged in observational as well as active motor learning. Although anodal tDCS over PM does not affect overall M1 excitability assessed by TMS thresholds for motor-evoked potentials or by TMS input/output functions, it does decrease short-interval intracortical inhibition and increase intracortical facilitation in M1, effects argued to result from activation of physiological connections from PM to M1 (Boros *et al*., 2008). Two other observations are relevant to the temporal and spatial location of the current finding. First, anodal tDCS over PM during physical practice of an SRTT has been shown not to affect the rate of learning (Nitsche et al., 2003), indicating that the result reported here is likely due to an effect in the observational phase. Second, while it is clear that anodal tDCS applied over M1 during physical practice increases the rate of learning of both implicit (Nitsche *et al*., 2003) and explicit (Stagg *et al*., 2011) finger-sequencing tasks, identical stimulation applied immediately before physical practice has no effect on subsequent learning (Kuo et al., 2008) or even retards subsequent learning (Stagg et al., 2011). These observations, together with the other evidence implicating PM in observational motor learning, converge with the current findings to support the conclusion that increased excitability of PM before physical practice was the critical factor in enhancing the effects of observation.

It might be argued that subjects made covert finger movements during the observation phase, and thus the observed effect of anodal tDCS during this phase could be attributed to increased M1 excitability on neural representations formed by physical practice. The possibility that observational learning of motor skills results from covert physical movements has been considered in previous work and has been ruled out by two main findings. First, observational learning of a motor skill is independent of the effector observed, indicating that observation creates a general motor representation and not a specific effector-linked motor representation (Buchanan & Wright, 2011; Hayes, *et al*., 2010; Williams & Gribble, 2012). Second, and more directly, observational learning of a reaching movement in a novel force field was shown not to be accompanied by any detectable electromyographic activity in recordings from muscles shown to be active during physical performance of the movement (Mattar & Gribble, 2005). Together, these findings are strong evidence that observational learning of motor skills is not a result of covert activation of the relevant effectors during observation. The consensus in the literature that observational learning does not depend on covert physical practice of the skill supports the conclusion that anodal tDCS acted on an effector-independent representation formed in the action-observation network during observation.

When applied close together in time, two or more techniques that alter the excitability of motor cortex can recruit homeostatic-like mechanisms that limit the amount and direction of plastic change in excitability, and so act to maintain excitability within a physiological range (see reviews by Ziemann & Siebner, 2008, and Ridding & Ziemann, 2010). Neurophysiological evidence of homeostatic-like regulation of motor cortical excitability has been shown with anodal tDCS in combination with other excitatory neuromodulatory techniques (Lang et al., 2004; Siebner et al., 2004). In the present experiment, there was no evidence of a homeostatic interaction between the three putative excitatory processes, observation of the ordered sequence ,anodal tDCS during the observation phase, and subsequent physical practice of the movement sequence. This may have been because observation had only a small and transitory excitatory effect which was evident behaviorally only early in physical practice. This view is consistent with a behavioral assessment of homeostatic plasticity which showed that anodal tDCS over M1 immediately before physical practice of an SRTT (which is known also to increase M1 excitability) did not recruit homeostatic regulation of plasticity (Kuo et al., 2008). These authors found evidence of homeostatic plasticity only in a group that was given anodal tDCS over M1 in combination with another excitatory manipulation, pre-treatment with a partial NMDA receptor agonist.

There has been considerable interest in the use of anodal tDCS as an adjunctive therapy to facilitate functional rehabilitation following brain injury, with some evidence that it can promote recovery of upper-limb function (see reviews by Brunoni *et al*., 2012; Vallar & Bolognini, 2011). Attention has been drawn recently to using action observation to promote motor rehabilitation (Sale & Franceschini, 2012; Small *et al*., 2012; Small *et al*., 2013). More specifically, Small *et al*. (2013) have proposed ‘action observation treatment’, in which action observation is followed by action execution, as an effective strategy for neurorehabilitation. The present finding that the effects of motor observation on subsequent motor sequence learning can be potentiated by increasing the excitability of PM during the observation phase could increase the effectiveness of this strategy. Moreover, action observation alone with anodal tDCS might be an effective therapeutic strategy for motor rehabilitation in those who are limited in their ability to engage in intensive physical therapy.

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Abbreviations:

AON: action observation network; M1: primary motor cortex; PM: premotor cortex; SRTT: serial reaction time task; tDCS: transcranial direct current stimulation; TMS: transcranial magnetic stimulation; *M*: mean; *SEM*: standard error of the mean.

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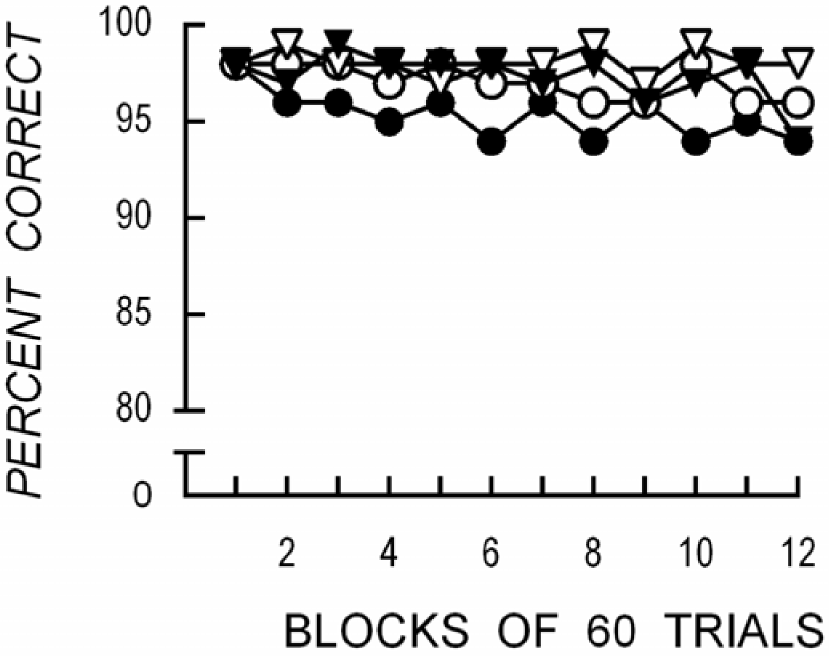
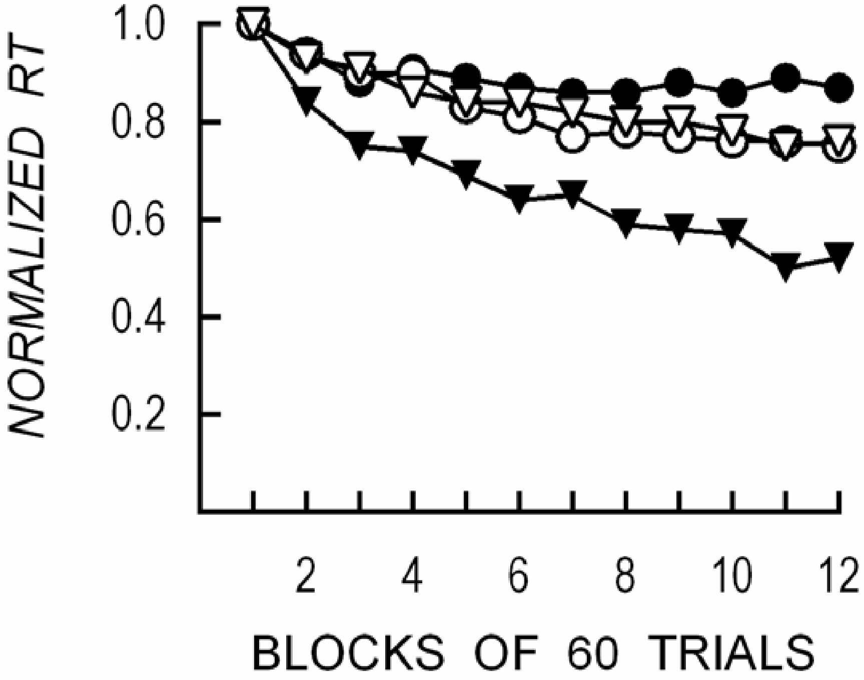
Figure Legends

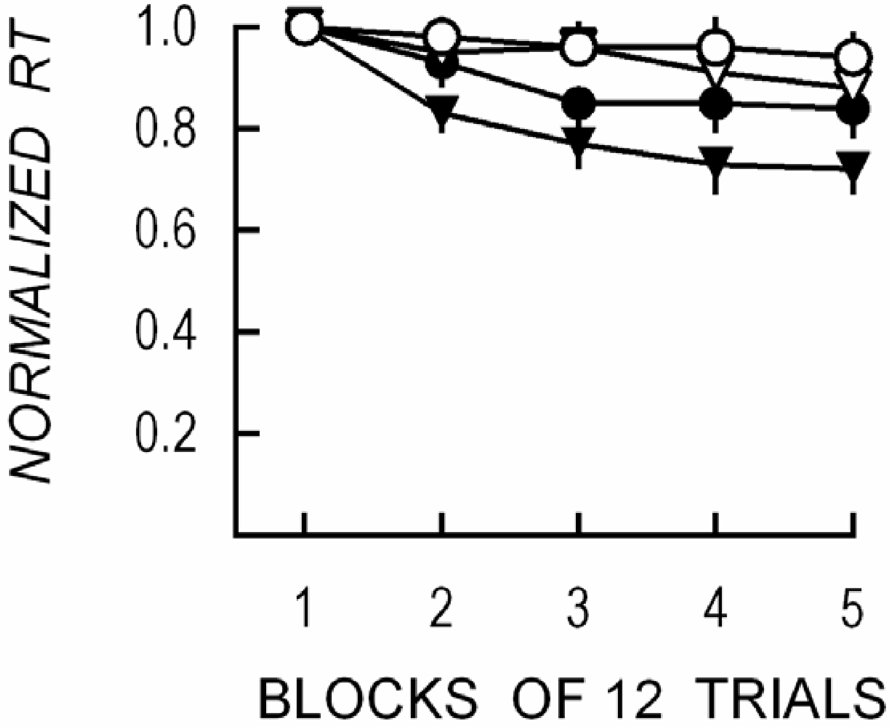
Figure 1. Mean accuracy (percent correct) for each group (tDCS/ordered: filled triangles; tDCS/irregular: open triangles; sham/ordered: filled circles; sham/irregular: open circles) for each block of 60 trials. Error bars are not presented for the purpose of clarity.

Figure 2. Mean normalized reaction times for each group (tDCS/ordered: filled triangles; tDCS/irregular: open triangles; sham/ordered: filled circles; sham/irregular: open circles) for each block 60 trials. Error bars are not presented for the purpose of clarity.

Figure 3. Mean normalized reaction times for each group (tDCS/ordered: filled triangles; tDCS/irregular: open triangles; sham/ordered: filled circles; sham/irregular: open circles) for the first 60 trials in blocks of 12 trials. Unidirectional error bars show 1 SEM.


PERCENT CORRECT
100
95
90
85
80
0
2
4
6
8
10
12
BLOCKS OF 60 TRIALS


NORMALIZED RT
1.0
0.8
0.6
0.4
0.2
2
4
6
8
10
12
BLOCKS OF 60 TRIALS


NORMALIZED RT
1.0
0.8
0.6
0.4
0.2
1
2
3
4
5
BLOCKS OF 12 TRIALS