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Title: Online and offline effects of cerebellar transcranial direct current stimulation on motor learning in healthy older adults: A randomized double-blind sham-controlled study

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Abstract:

The aim of this randomized double blinded sham-controlled study was to determine the effect of cerebellar anodal transcranial direct current stimulation (a-tDCS) on online and offline motor learning in healthy older individuals. Thirty participants were randomly assigned in experimental (n=15) or sham tDCS (n=15) groups. Participants in experimental group received 2 mA cerebellar a-tDCS for 20 minutes. However, the tDCS was turned off after 30 seconds in sham group. Response time (RT) and error rate (ER) in serial response time test were assessed before, during 35 minutes and 48 hours after the intervention. Reduction of RT and ER following the intervention session was considered as short-term (35 minutes post intervention) and long-term offline learning (48 hours post intervention) respectively. Online RT and ER reduction were similar in both groups ($p>0.05$). RT was significantly reduced 48 hours post intervention in cerebellar a-tDCS group ($p=0.03$). Moreover, RT was significantly increased after 35 minutes and 48 hours in sham tDCS group ($p=0.03$, $p=0.007$), which indicates a lack of short-term and long-term offline learning in older adults. A-tDCS on cerebellar region produced more short-term and long-term offline improvement in RT ($p=0.014$, $p=0.01$) compared to sham tDCS. In addition, online, short-term and long-term (48 hours) offline error reduced in cerebellar a-tDCS as compared to sham-control group, although

this reduction was not significant ($p>0.05$). A deficit suggests that a direct comparison to a younger group was made. The findings suggested that cerebellar a-tDCS might be useful for improvement of offline motor learning in older individuals.

Introduction:

Ageing population is growing rapidly worldwide (De Luca *et al.*, 2010) and face countries with growing age related problems. Older adults show decline in the performance of daily living activities (Park *et al.*, 2001; Bohannon *et al.*, 1984; Holviala *et al.*, 2012; Laughton *et al.*, 2003; Prince *et al.*, 1997) which in turn can affect the quality of their lives. In this regard, development of novel approaches to control these changes (Zimmerman & Hummel, 2010) and improve the quality of life in this population is very important (Hall *et al.*, 2011).

Aging is associated with brain changes that can limit its functional capacity (Lustig *et al.*, 2009). It appears that this neural functional changes have been associated with performance declines in daily activities in older adults (Reuter-Lorenz & Park, 2010; Seidler *et al.*, 2010). Substantial evidence indicated that the cerebellum is an important region for the formation of internal models of behavior, the timing of behaviors (Keele & Ivry, 1990), and the sequence processing of learning (Molinari & Leggio, 2013). Some studies showed decreased activity in cerebellum in older adults compared to younger adults (Bernard & Seidler, 2013; Bernard & Seidler, 2014). Since, the cerebellum and other brain areas such as basal ganglia are playing important roles in timing and sequencing of behaviors (Keele & Ivry, 1990; Molinari & Leggio, 2013), observation of timing and sequence learning deficits in the older adults may suggest contribution of the cerebellum in the age-related performance declines (Bernard & Seidler, 2013; Baudouin *et al.*, 2006; McCormack *et al.*, 2002; Bo *et al.*, 2009; Bo *et al.*, 2011). In addition, changes in morphology, stereology and volume of cerebellum by aging have been shown in previous studies (Keele & Ivry, 1990; Bernard & Seidler, 2013; Andersen *et al.*, 2003; Tang *et al.*, 2001). Therefore, enhancement of cerebellar functions could be used to reduce age-related decline in motor and cognitive functions of the older age individuals.

Cerebellar anodal transcranial direct current stimulation (a-tDCS) is a non-invasive technique for induction of prolonged functional changes in the cerebellum (Ferrucci *et al.*, 2015). The effects of cerebellar a-tDCS have already been shown in modulation of cerebello-cortical

connections (closed-loop connectivity between prefrontal or primary motor cortices and the cerebellum) and therefore neurophysiological and behavioral changes (Chen *et al.*, 2014; Galea *et al.*, 2009), gait adaptation (Jayaram *et al.*, 2012), motor learning (Herzfeld *et al.*, 2014; Galea *et al.*, 2011; Hardwick & Celnik, 2014; Shah *et al.*, 2013; Ehsani *et al.*, 2016) and cognition (Ferrucci *et al.*, 2013, Ferrucci *et al.*, 2012) in healthy humans.

Some studies assessed the effect of a-tDCS in different cortical areas in healthy older individuals and demonstrated improvement of motor skills and cognitive functions in this population (Hummel *et al.*, 2010; Zimmerman *et al.*, 2013; Holland *et al.*, 2011; Berryhill & Jones, 2012; Meinzer *et al.*, 2013). Although many studies demonstrated that cerebellum is considerably affected by aging (Bernard & Seidler, 2014; Baudouin *et al.*, 2006; McCormack *et al.*, 2002; Bo *et al.*, 2009; Bo *et al.*, 2011), only one study evaluated the cerebellar a-tDCS effect on motor learning in older adults (Hardwick & Celnik, 2014). In this study, the authors demonstrated that cerebellar a-tDCS could enhance motor adaptation and reduce error rate during a visuomotor task in older adults (Hardwick & Celnik, 2014). This study indicated significant error reduction in older adults who received cerebellar a-tDCS as compared to sham condition during the adaptation phase of learning (online learning) and post-adaptation after effects (short-term offline learning) (Hardwick & Celnik, 2014). However, they did not assess the lasting effects of this intervention (Hardwick & Celnik, 2014). In addition, they evaluated cerebellar a-tDCS effects during adaptation learning (Hardwick & Celnik, 2014). Whereas, adaptation learning and sequence learning induce different contributions of brain regions in different stages of learning process and can have different effects during cerebellar tDCS in older adults (Doyon & Benali, 2005).

The purpose of the present study was to assess the effect of cerebellar a-tDCS on the response time (RT) and error rate (ER) during and after completion of a motor sequence task. We hypothesized that cerebellar a-tDCS would increase the offline motor sequence learning in the older individuals.

Method and materials:

Participants:

Thirty healthy older adults participated in this study. This sample size allows detecting the effect of cerebellar a-tDCS (power of 85%) with 95% confidence interval (CI). The flowchart of eligibility assessment throughout the study is presented in Figure 1. Thirty-six volunteers were screened against the inclusion and exclusion criteria of the study by a physician. However, 4 of them did not meet the criteria and just 32 healthy individuals were randomly assigned into two groups: cerebellar or sham a-tDCS groups. Two participants (one from each group) withdrew from the study before receiving the intervention. Twenty-three women and 7 men with mean age of 68.70 ± 5.28 completed the study. The inclusion criteria were being older than 60 years with no history of neurological or musculoskeletal disorders. The exclusion criteria were: having severe perceptual and memory problems [scores of less than 21, assessed by Mini Mental Status Examination (MMSE)]; suffering from any neurological disease, suffering from any musculoskeletal or rheumatoid condition affecting range of motion in upper extremity and any contraindications for having tDCS e.g. intracranial metal implantation.

All participants were right-handed, as determined by the Edinburgh Handedness Inventory (10 item version)(Light & Singh, 1987). All participants signed a written informed consent before participation in the study. This study met the criteria in CONSORT checklist.

<<< Please insert Figure 1 near here>>>

Study design:

The design of this study was randomized, double blinded, sham-controlled study. Participants (n=15 in the cerebellar a-tDCS group and n=15 in the sham tDCS group) received a-tDCS or sham tDCS and performed serial response time test (SRTT) training concurrently. RT and the number of errors were measured before, immediately, 35 minutes and 48 hours after the completion of experimental conditions.

The study was approved by Human Ethics Committee at the Semnan University of Medical Sciences, Semnan, Iran, which is associated with declaration of Helsinki. The study was registered as a clinical trial study on Iranian Registry of Clinical Trials (The registration number: IRCT2015112321294N2, www.irct.ir).

Tool for induction and assessment of motor learning

A custom designed software program known as Color Matching Test (CMT) was used to induct and assess the motor learning in this study. This software program emulated a SRTT condition with second-order structural pattern (Ehsani *et al.*, 2015). SRTT is one of the most common methods for assessing implicit motor learning which integrates both motor and cognitive components (Nissen & Bullemer, 1987). In CMT software, colored squares repeatedly appeared in the center of a computer screen. A specific key on the keyboard was assigned for each color square (yellow: left shift key; red: C; green: M; and blue: right shift key). As soon as the correct key was pressed by the participant, the next colored square was appeared. All participants were instructed to use their right index finger to press the keys.

All participants were completed 2 blocks as a pre-test, 8 blocks as the main training task and another 2 blocks in 35 minutes and 48 hours after completion of the main training. The second block of the pre-test was considered as the baseline data. The 2 blocks after the main training task were used to assess short-term (35 minutes post training) and long-term (48 hours post training) offline learning effects (Fig. 2).

The main training task includes 8 blocks with one-minute rest between them. Unlike the blocks 5 and 6 which had a random pattern, the other six blocks presented with ordered pattern (Fig. 2). The participants became familiar with the instruction of the SRTT, prior to the test. An ordered block included 10 trials and each trial contained 8 color cues in a particular sequence (i.e. Yellow-Red-Green-Yellow-Blue-Green-Yellow-Green.....YRGYBGYG) (Stagg *et al.*, 2011). Each random block also included 10 trials, but the color cues of each trial were randomly presented (i.e. RBGYBRYG.....GBRYYBGR) (Fig. 2). The mean of the RT and the number of errors for the first and the last block of the main training task were used for analysis of online learning (within-session). Two ordered blocks were applied 35 minutes and 48 hours after completion of the main training task to assess short-term and long-term offline learning effects (Fig. 2).

To assess whether the participants could explicitly recognize the order of sequences and obtained explicit knowledge (Ehsani *et al.*, 2015); they were asked if they could remember the

exact sequence of the ordered trial. If any participant could remember the exact sequence of the ordered trial, his data was not included in data analysis.

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Transcranial direct current stimulation

The tDCS device (tDCS Brain Stimulation Device - ApeX Type A, USA Model, China) was utilized to deliver 2 mA direct current for 20 minutes (10 sec ramp-up/down) (Nitsche & Paulus, 2000; Brunoni *et al.*, 2011; Galea *et al.*, 2009). The size of the active and returning electrodes was 5×5 cm. In the experimental group, the active anode electrode was placed over ipsilateral cerebellum (1 cm belowinion of occipital bone and 1 cm medial to mastoid process). The returning electrode was placed over right arm (Ferrucci *et al.*, 2008; Ferrucci *et al.*, 2013; Ehsani *et al.*, 2016) (Fig.3). In the sham-controlled group, the electrode montage was similar to the experimental group, however the tDCS was turned off after 30 seconds of stimulation.

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Experimental procedures

Participants were asked to sit in front of a computer monitor. All participants were instructed to hit the assigned key for a particular color with their right index finger as soon as they see the color. They received tDCS during the main training session (Hardwick & Celnik, 2014). Both participants and assessors were blinded to the experimental conditions (active or sham a-tDCS). Blinding integrity was checked at the end of the training session by asking the participant if they could recognize the nature of the condition (active or sham stimulation).

Assessment of the side effects

All participants were monitored for any side or adverse effects related to the tDCS interventions. They were asked to complete a questionnaire after the tDCS intervention. This questionnaire determined the presence and severity of possible side effects such as itching, tingling, burning sensations under electrodes (George & Aston-Jones, 2010; Nitsche *et al.*,

2008) and also headache and pain during and after the stimulation. The questionnaire included rating scales; numeric analogue scales (e.g., 0= no tingling to 10= worst tingling imaginable).

Operational Definitions

The RT and the ER were the main variables to assess motor learning in this study. The RT is defined as the “time from the presentation of the color to the key press” and includes both reaction and movement time. The ER was defined as the mean of error numbers for each block. Online learning is the learning, which happening during the training session. Any reduction in the RT and/or ER at the block 10 (T_{10}) compared to the block 3 (T_3) were considered as online learning (Reis *et al.*, 2009). Short- and long-term offline learnings were considered as the lasting effect of learning that happened after the completion of the training (35 minutes and 48 hours post intervention, respectively). In this regard, short-term offline learning was defined as any decrease in the RT or ER of block 12 (T_{12}) compared to block 10 (T_{10}). Similarly, long-term offline learning was defined as any decrease in the RT or ER of block 14 (T_{14}) compared to block 10 (T_{10}).

Data analysis

SPSS software, version 22, was used to analyze the data. Shapiro-Wilk test was conducted to evaluate the normality of distribution for tested variables. Normal distribution was observed for all variables in the groups. An independent sample t-test was also performed to test lack of difference in the baseline values between groups. A two-way repeated measure ANOVA was used to assess the main effects of “Group” (cerebellar and sham a-tDCS), “Time” (T_2 , T_3 , T_{10} , T_{12} and T_{14}) and their interactions on the RT. Similarly, a two-way repeated measure ANOVA was conducted to evaluate the errors during training and post training among groups. In addition, an independent sample t-test was used to compare online and offline learning between groups. Post-hoc tests with Bonferroni correction were performed where indicated. Type I error (α) was set at 0.05 and the power of tests was considered 0.85.

Results:

Demographic characteristics

The demographic details and baseline data for the participants in each group are presented in Table 1. There were no significant differences between groups in regards to age, gender, MMSE, baseline RT or errors ($P > 0.05$). In addition, the means of RT and error number of essential blocks for both groups are demonstrated in Table 2. Moreover, the results indicated that any of the participants could not explicitly remember the pattern of ordered blocks. Therefore all the data were analyzed.

The effect of a-tDCS on RT

The results of two-way repeated measures ANOVA are shown in Table 3. The between-subjects main effect of “Group” was significant ($P < 0.001$), which indicated differences in the learning between groups. The within-subjects main effect of “Time” was also significant ($P < 0.001$), which indicated significant differences between degrees of learning during different time points in each group (i.e. block 2, 3, 10, 12 and 14). ANOVA also showed that there was significant interaction effect between “Group” and “Time” ($P = 0.02$), which indicated significant differences between groups at some time points.

Post hoc analysis using Bonferroni correction showed significant RT decreases between T_3 and T_{10} and between T_{10} and T_{14} , second retention follow-up (48 hours) in cerebellar a-tDCS group ($P < 0.05$) (Table 4) (Fig. 4A). In sham group, there were significant RT decreases between T_3 and T_{10} ($P < 0.05$), while there were significant increasing RT between T_{10} and T_{12} or between T_{10} and T_{14} ($P < 0.05$), which indicated deficit in short-term and long-term offline learning in sham controlled group (Table 4) (Fig. 4B). Moreover, there were significant differences in short-term ($P = 0.014$), and long-term (48 hours) offline RT reduction ($P = 0.001$) between groups (Fig. 5C and E), while there was no significant difference in online learning between groups ($P > 0.05$) (Fig. 5A).

The effect of a-tDCS on ER

The ER was also assessed for each block in the study, which indicated significant within and between-subject main effects ($P < 0.001$) (Table 3). ANOVA also showed significant interaction effect between “Group” and “Time” points ($P < 0.001$). There was significant errors

reduction between T₃ and T₁₀ ($P < 0.05$), while there were no significant decreases between T₁₀ and T₁₂ ($P < 0.05$), or between T₁₀ and T₁₄ ($P > 0.05$) in cerebellar a-tDCS group (Table 4 and Fig. 4C). In sham-control group, the errors were reduced significantly between T₃ and T₁₀ ($P < 0.05$). However, the errors did not change from T₁₀ to T₁₂ or T₁₀ to T₁₄ in this group ($P > 0.05$) (Table 4 and Fig. 4D). In addition, the error reduction for online, short-term and long-term (48 hours) offline learning period was not significant in cerebellar a-tDCS compared to sham-control group ($P < 0.05$) (Fig. 5B, D and F).

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Safety and side effects of a-tDCS

All participants tolerated the cerebellar a-tDCS intervention very well with minimal adverse or side effects. Table 5 summarizes reported side effects (means $\pm$ SEM) under the anode and cathode for each group. Itching was the common side effect of a-tDCS, which was reported by the most participants in this study during stimulation period. However, they did not report any burning sensation, or pain during stimulation. Moreover, no side effects were reported by the participants after the end of stimulation.

Discussion:

This study shows significant differences in short-term and long-term offline learning between the active and sham tDCS groups. The aim of the current study was to assess the effects of cerebellar a-tDCS on online learning and its lasting effects on sequenced motor learning in healthy older adults. The findings indicated that although participants in sham group could achieve considerable performance improvement during the training session (online learning), they could not maintain the offline component of motor sequence learning. In addition, the results revealed that using a-tDCS over cerebellum could increase online learning and also significantly improve short-term and long-term offline learning in healthy older individuals. This finding is mainly evidenced by reduction in RT.

We hypothesized that cerebellar a-tDCS increases offline learning in older adults. The findings of the present study supported this hypothesis and indicated that those participants who received cerebellar a-tDCS showed short-term and long-term offline learning as

compared to sham group. Whereas, the participants in sham group could not maintain the short-term or long-term offline RT and ER reduction. In this regard, some studies showed that older adults could have online motor learning, however they could not maintain this learning effect after the training (Bo *et al.*, 2011; Ehsani *et al.*, 2015; Howard *et al.*, 2004). In addition, the findings of this study are supported by findings of some studies that assessed the cerebellar a-tDCS effects on offline learning of healthy young adults (Herzfeld *et al.*, 2014; Galea *et al.*, 2011; Ferrucci *et al.*, 2015; Galea *et al.*, 2009; Ehsani *et al.*, 2016). Ehsani *et al.* (2016) reported that cerebellar a-tDCS could be used as an effective technique for improvement of offline learning in healthy individuals and cerebellum has key role in processing of implicit motor learning up to 48 hours in healthy younger individuals (Ehsani *et al.*, 2016). Herzfeld *et al.* (2014) assessed the lasting effects of cerebellar a-tDCS on motor learning for up to 24 hours in healthy younger adults and reported that the cerebellar a-tDCS had lasting effect on motor memory in younger individuals (Herzfeld *et al.*, 2014).

In line with Hardwick & Celnik (2014) study, the current study indicated that short-term offline motor learning in older adults could be improved by cerebellar a-tDCS (Hardwick & Celnik, 2014). In addition, the current study also indicated significant improvement in induction of the long-term offline motor learning in older adults. It appears that cerebellum is a critical region for procedural learning and application of a-tDCS over the cerebellum could produce lasting changes on motor learning in healthy population (Herzfeld *et al.*, 2014; Galea *et al.*, 2009; Galea *et al.*, 2011). The finding of current study showed that a-tDCS of the cerebellum could be used in healthy older individuals to compensate the aging-related learning potentials.

A-tDCS of cerebellum may enhance functional activity of cerebellum and cerebello-cortical interaction (Galea *et al.*, 2009; Hamada *et al.*, 2012; Celnik, 2015), which may deficit by aging (Bernard & Seidler, 2013; Bernard & Seidler, 2014). In addition, it could be argued that cerebellar a-tDCS may facilitate implicit motor learning by improving cognitive abilities (Galea *et al.*, 2011; Ferrucci *et al.*, 2013; Pope & Miall, 2012). So, it can improve cognitive and motor behaviors in adults (Ferrucci *et al.*, 2013). The benefits of cerebellar a-tDCS on the memory formation have shown in a number of studies (Ferrucci *et al.*, 2008; Ferrucci *et al.*, 2013; Boehringer *et al.*, 2012). In this regard, Hardwick & Celnik reported enhancing

encoding and consolidation of memory formation in older adults during procedural learning with using the cerebellar a-tDCS (Hardwick & Celnik, 2014). On the other hand, given the preferential effects of cerebellar a-tDCS on offline learning, it seems that the a-tDCS effects act on consolidation and possibly retrieval processes. Lasting changes on motor learning and retrieving memory formation for cerebellar a-tDCS have been shown in a number of studies (Herzfeld *et al.*, 2014; Galea *et al.*, 2009; Galea *et al.*, 2011; Ehsani *et al.*, 2016). In addition, our study indicated that cerebellar a-tDCS potentially induced lasting effect on RT reduction more than ER reduction. It seems that the cerebellum is essential in temporal relationship and spatial realignment during serial tasks and between successive events (Ivry R, 1997; Hardwick & Celnik, 2014). Accordingly, cerebellar modulation also may enhance the cerebellar function in temporal and spatial realignment that affect reduction of RT more than ER (Ivry R, 1997).

In line with the findings in current study, cerebellar a-tDCS should be applied concurrently with the training task to induce significant changes (Herzfeld *et al.*, 2014; Galea *et al.*, 2009; Galea *et al.*, 2011; Ferrucci *et al.*, 2013). This conclusion was also confirmed by the findings of the other studies which indicated that this improvement could not be achieved by application of cerebellar a-tDCS prior to the training task (Kuo *et al.*, 2008; Stagg *et al.*, 2011).

Safety and side effects of a-tDCS

The results of the current study indicated that cerebellar a-tDCS technique is a safe method with minimal side effects and no adverse effect in healthy older individuals. Itching and/or tingling were reported as the common side effects of active and sham tDCS by older participants.

Limitations of the study

In this study, 77% of participants were older females, which limit the extrapolation of these findings to the male or younger population. In addition the sample size was small which may also affects the extrapolation of the findings to a larger population. Another limitation of the study worth mentioning is the lack of neurophysiological measures in any kind (fMRI, cortical

excitability). These assessments would be useful to comment on the possible mechanisms behind these observations.

Suggestions for future research

Our study assessed the effects of only one session application of cerebellar a-tDCS on motor learning in the older adults. Multiple tDCS application might be more effective in motor learning in this population. In addition, further studies are recommended to investigate the lasting effect of the cerebellar a-tDCS with longer follow ups in older population. Moreover, further studies are suggested to compare the lasting effects of cerebellar a-tDCS on motor learning between young and older adults. Investigation of gender effect is also important, which requires further attention. Using more localized electrodes is also suggested in future studies.

Conclusion:

The findings of current study indicate that cerebellar a-tDCS increases short-term and long-term offline motor learning in older adults. The findings indicate that cerebellum has a key role in processing of implicit motor learning of older adults and cerebellar a-tDCS may induce changes in motor learning which may last up to 48 hours in healthy older individuals.

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Author contributions

All authors; Fatemeh Ehsani, Afshin Samaei, Maryam Zoghi, Mohaddese Hafez Yosephi and Shapour Jaberzadeh; substantially contributed to the i) conception or design of the work; or

the acquisition, analysis, or interpretation of data for the work; ii) drafting the work or revising it critically for important intellectual content; iii) final approval of the version to be published and iv) agreement to be accountable for all aspects of the work.

Competing interests

There was no conflict of interest.

Abbreviations

a-tDCS, anodal transcranial direct current stimulation; ER, error rate; CI, confidence interval; CMT, Color Matching Test; MMSE, Mini Mental Status Examination; RT, response time; SRTT, serial response time test.

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Figure captions:

Figure 1. Flow diagram of participant's eligibility assessment

Figure 2. A flow diagram of the testing conditions for comparing the effect of cerebellar a-tDCS on the reaction time and the number of errors of serial response time test training.

Figure 3. Electrode montages for a-tDCS over cerebellum. Active electrode was placed over ipsilateral cerebellum (1 cm below inion of occipital bone and 1 cm medial to mastoid process) and the returning electrode was placed over right arm.

Figure 4. **a)** Response time (Mean $\pm$ SEM) of blocks during serial response time test training in cerebellar tDCS group, **b)** Response time (Mean $\pm$ SEM) of blocks during serial response time test training in cerebellar sham tDCS group, **c)** Errors number (Mean $\pm$ SEM) of blocks during serial response time test training in cerebellar tDCS group, **d)** Errors number (Mean $\pm$ SEM) of blocks during serial response time test training in cerebellar sham tDCS group. SEM: standard error of measurement.

Figure 5. **a)** The comparison of online learning (changes in response time and errors number between blocks 3&10) (Mean differences $\pm$ SEM) among groups, **b)** The comparison of short-term offline learning (changes in response time and errors number between blocks 2&12) (Mean differences $\pm$ SEM) among groups, **c)** The comparison of long-term offline learning (changes in response time and errors number between blocks 2, 14) (Mean differences $\pm$ SEM) among groups; *indicates significance difference. SEM: standard error of measurement.

Table1: Demographic data and baseline values of the participants in groups (Mean±SEM)

Variable	Group (Mean±SEM)		
	Cerebellar	Sham	P value
	a-tDCS	tDCS	
Age (year)	69.40± 5.08	68± 5.55	0.48
Gender (Male/Female)	4/11	3/12	0.68
MMSE Test	24.73±1.44	25.07±1.67	0.56
Time of block 2	61.60±1.99	62.87±2.01	0.81
Error numbers of block 2	3.87±0.38	3.67±0.47	0.74

MMSE: Mini Mental Status Examination; SEM: Standard error of measurement

Table 2: Response time and errors rate (Mean± SEM) for important blocks of serial response time test in each group

Variable	Group (Mean± SEM)
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		Cerebellar a-tDCS	Sham tDCS
Block			
2	Response time	61.60±2.43	62.87±2.62
	Error rate	3.87±0.36	3.67±0.77
3	Response time	61.20±1.76	62.60±1.56
	Error rate	3.67±0.47	3.60±0.64
10	Response time	42.73±1.93	47.87±1.98
	Error rate	1.6±0.49	2.33±0.43
12	Response time	38.73±1.21	51.47±1.58
	Error rate	1.80±0.21	2.60±0.47
14	Response time	36.74±1.11	53.33±1.34
	Error rate	1.20±0.23	2.86±0.21

SEM: Standard error of measurement

Table 3: ANOVA results for the effects of a-tDCS on response time and errors rate

Outcome Main and interaction effects of ANOVA		Measures	Df	F	P value
Response time	Time (within-subject effects)		4	33.26	<0.001*
	Group*Time (interaction effects)		4	4.64	0.02*

	Group (between-subject effects)	1	1816.63	<0.001*
Errors number	Errors number (within-subject effects)	4	13.99	<0.001*
	Group*Errors number (interaction effects)	4	2.46	<0.001*
	Group (between-subject effects)	1	406.01	<0.001*

Table 4: Post-hoc pair-wise comparison of response time and error rate between the blocks in each group

Group	Variables	Block	Block	Mean differences (95% CI)	P value
Cerebellar a-tDCS	Response time	3	10	-18.47 (-20.14- -13.66)	<0.001*
		10	12	-4.00 (-9.32- -1.32)	0.13
		10	14	-6.00 (-11.36- -0.64)	0.03*
	Error rate	3	10	-2.07 (-2.32- -1.68)	0.001*

Sham tDCS		10	12	-0.20 (-1.02- -0.62)	0.61
		10	14	-0.40 (-1.02- -0.22)	0.19
	Response time	3	10	-14.73 (-17.84- -11.63)	0.013*
		10	12	3.60 (0.41-6.79)	0.03*
		10	14	5.47 (1.78-9.15)	0.007*
	Error rate	3	10	-1.27 (-1.95- -0.18)	0.012*
		10	12	0.27 (0.85-1.38)	0.62
		10	14	0.53 (0.33-1.39)	0.21

Table 5. Numeric sensation score by participants during experimental conditions

	Anode electrode		Cathode electrode	
	Cerebellar	Sham tDCS	Cerebellar	Sham tDCS
	a-tDCS		a-tDCS	
Tingling sensation	2.2±0.27	0.7±0.19	0.65±0.15	0.54±0.21
Itching	1.9±0.19	0.3±0.04	0.81±0.21	0.27±0.08

sensation				
Burning sensation	-	-	-	-
Not tolerated	-	-	-	-

Numeric Analogue Scale (NAS); 0 is rated as no sensation and 10 rated as the worst sensation imaginable; was used to rate the values of side effects. The participants reported their sensations under both active (anode) and reference (cathode) electrodes after cerebellar a-tDCS and sham tDCS intervention. Scores are reported as mean $\pm$ SEM (SEM: Standard error of measurement).

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TDCS OF CEREBELLUM:

Montage:

Extra cephalic

Active electrode (Anode):

Over ipsilateral cerebellum (5 x 5 cm)

Return electrode (Cathode):

Over ipsilateral shoulder (5 x 5 cm)

Intensity:

2 mA

Density:

0.08 mA/Cm²

Duration of application:

20 minutes

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