HHS Public Access

Author manuscript

Cerebellum. Author manuscript; available in PMC 2021 June 01.

Published in final edited form as:

Cerebellum. 2020 June ; 19(3): 383–391. doi:10.1007/s12311-020-01114-w.

POLARITY- AND INTENSITY-INDEPENDENT MODULATION OF TIMING DURING DELAY EYEBLINK CONDITIONING USING CEREBELLAR TRANSCRANIAL DIRECT CURRENT STIMULATION

Jessica Mitroi[*,1], **Leah Burroughs**[*,1], **Alexandra B. Moussa-Tooks**[1,2], **Amanda R. Bolbecker**[1,3], **Nancy B. Lundin**[1,2], **Brian F. O'Donnell**[1,2,3], **William P. Hetrick**[1,2,3]

[1]Psychological & Brain Sciences, Indiana University, Bloomington, IN

[2]Program in Neuroscience, Indiana University, Bloomington, IN

[3]Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN

Abstract

Background: Delay eyeblink conditioning (dEBC) is widely used to assess cerebellar-dependent associative motor learning, including precise timing processes. Transcranial direct current stimulation (tDCS), noninvasive brain stimulation used to indirectly excite and inhibit select brain regions, may be a promising tool for understanding how functional integrity of the cerebellum influences dEBC behavior.

Objective/Hypothesis: The aim of this study was to assess whether tDCS-induced inhibition (cathodal) or excitation (anodal) of the cerebellum impairs or facilitates, respectively, timing of dEBC.

Methods: A standard 10-block dEBC paradigm was administered to 102 healthy participants. Participants were randomized to stimulation conditions in a double-blind, between-subjects sham-controlled design. Participants received 20-minute active (anodal or cathodal) stimulation at 1.5mA (n=20 anodal, n=22 cathodal) or 2mA (n=19 anodal, n=21 cathodal) or sham (n=20) stimulation concurrently with dEBC training. Stimulation intensity and polarity effects on percent conditioned responses (CRs) and CR peak and onset latency were examined using repeated measures analyses of variance.

Corresponding Author: William Hetrick, PhD, 1101 E. 10th St., Bloomington, IN, 47405, Phone: (812) 855-2620, Fax: (812)855-4691, whetrick@indiana.edu.

*Both authors contributed equally to this study and manuscript

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Indiana University Institutional Review Board protocol #1508694422) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent: Informed consent was obtained from all individual participants included in the study.

Results: Acquisition of CRs increased over time at a similar rate across sham and all active stimulation groups. CR peak and onset latencies were later, i.e., closer to air puff onset, in all active stimulation groups compared to the sham group.

Conclusion(s): tDCS facilitated cerebellar-dependent timing of dEBC, irrespective of stimulation intensity and polarity. These findings highlight the feasibility of using tDCS to modify cerebellar-dependent functions and provide further support for cerebellar contributions to human eyeblink conditioning and for exploring therapeutic tDCS interventions for cerebellar dysfunction.

Keywords

cerebellum; transcranial direct current stimulation; associative learning; eyeblink conditioning; polarity; intensity

Introduction

The cerebellum accounts for an estimated ten percent of the brain's volume [1] and contains at least half of the brain's neurons [2]. A large literature suggests that the cerebellum plays a dominant role in the coordination, fluidity, error correction, and timing of motor functions [3–10]. A prominent theory has arisen that the cerebellum is integral in the formation of internal models that are acquired, trained, and employed in the implicit prediction, expectation, and preparation of future events [11, 12], highlighting the importance of these timing functions in cerebellar processes. Nevertheless, little work has been done to systematically manipulate cerebellar function to demonstrate cerebellar contributions to distinct timing processes in humans.

Temporal Regulation of Cerebellar-Dependent Eyeblink Conditioning

One commonly and successfully used cerebellar-dependent task for understanding the neural mechanisms of associative learning is delay eyeblink conditioning (dEBC), a task in which a participant may acquire a conditioned response (e.g., anticipatory eyeblink) resultant from continued pairing of a visual or auditory (e.g., flash of light or bell) conditioned stimulus and unconditioned stimulus (e.g., shock or puff of air at the eye) [13]. dEBC is thought to principally depend on the cerebellum [14–16]. The circuitry that supports dEBC has been mapped through several pathways [17]. Animal and human studies show that dEBC is dependent on the functional integrity of the cerebellar cortex and the interpositus (IP) nucleus (animal: [18–20]; human: [20–23]). Long-term depression (LTD) at parallel fiber-Purkinje cell synapses and coincident activation of climbing fibers cause Purkinje cells to release their inhibition on the anterior IP nucleus, which is tonically inhibited by these Purkinje cells [24]. This process will only occur in a subset of Purkinje cells, tuned to signal eyelid closure, whereas a separate population of Purkinje cells signal eyelid opening [24]. The IP nucleus, now in long-term potentiation (LTP), is coincidently signaled by mossy and climbing fibers and projects neurons back into the brainstem, signaling the conditioned blink response via the red nucleus and facial nerve [24]. In addition to the precise timing of cellular signaling to elicit a response, the elicited response must be optimally timed to produce eyelid closure at an adaptive moment — when the aversive stimulus occurs. Response timing is captured by measures of onset and peak latencies of the conditioned

response. Typically, these latencies should shift closer to the unconditioned stimulus onset, though still occurring before it, as the cerebellum optimizes its response. In certain clinical populations, such timing parameters may be impaired [25–29]. The tight temporal coupling of these processes and the detail with which the dEBC circuit has been characterized makes dEBC an optimal task for evaluating the modulatory role of cerebellar circuits, specifically the timing of conditioned responses.

Transcranial Direct Current Stimulation

One promising method by which this modulation of cerebellar circuits can occur is transcranial direct current stimulation (tDCS) [30]. While the mechanisms of tDCS are not fully understood, studies have reported modulation of neuronal resting membrane potential, gamma-Aminobutyric acid (GABA) activity, N-methyl-D-aspartate (NMDA) receptor responsivity modification, and induction of prolonged neurochemical changes [30, 31]. Nevertheless, effective targeting of specific brain regions using tDCS is complicated by two major limitations: (1) the proximity of different brain regions to the scalp and (2) the varied orientation of cells. In the case of the cerebellum, few sub-regions lie close enough to the scalp to be targeted effectively with tDCS. Several studies have investigated electrode placement to optimize the electric field induced by tDCS [22, 30, 32]. In particular, Rampersad and colleagues [30] tested the effects of the strength and direction of the electric field in six different common tDCS configurations. A common cerebellar tDCS electrode configuration, with one electrode placed 3 cm lateral to the inion and the other placed over the ipsilateral buccinator, utilized in the current study, achieved a reasonably high electric field at the target site [30]. Taken together, this evidence suggests that tDCS may be able to modulate cerebellar circuits.

Studies have shown that cerebellar tDCS does not consistently impact the ability to *acquire* the conditioned response (CR) in dEBC [32–34]. When evaluating the effects of anodal, cathodal, and sham stimulation in 30 healthy controls (10/group), cerebellar tDCS initially showed a polarity-dependent effect on dEBC, wherein anodal stimulation facilitated acquisition of the conditioned response while cathodal impaired it as compared to sham [34], though these effects were not replicated in a follow-up study utilizing a similar sample [32]. However, Zuchowski and colleagues did find timing differences during the eye blink conditioning task, specifically anodal stimulation shortening mean CR onset and cathodal delaying it [33]. A third, larger study also found no polarity-specific effects on conditioning rates until further analyses were employed to separate participants into relevant subgroups, though potential changes in the timing of the conditioned response were not evaluated [33].

The current study utilized a larger sample size to investigate whether cerebellar tDCS has polarity- (anodal vs. cathodal) and intensity-dependent (1.5mA vs. 2mA) effects on the timing parameters of this task and better understand the best methods for tDCS efficacy. Based on previous studies [32, 34] and tDCS modeling suggesting that cerebellar tDCS is most effective in cortical cerebellar structures [30, 32] and is dependent on cell orientation [35], we expected that there would be no differences in CR acquisition during dEBC. This is because, as mentioned, dEBC is heavily modulated by deep cerebellar structures, and the cerebellum's heavily folded structure results in a cellular architecture with many

orientations. Moreover, although many cerebellar tDCS studies have utilized a 2mA intensity [36], we tested two different tDCS intensities (1.5mA and 2mA) in an effort to replicate previous studies and to further determine if a smaller dose is sufficient for modulating timing. Given the previous findings of Beyer and colleagues [32] and Rampersad and colleagues [30], we predicted that although the ability to generate a conditioned response would not be greatly impacted by tDCS, the timing of conditioned responses would be impacted. Specifically, we predicted that anodal stimulation would speed up conditioned response timing while cathodal would slow it down due to differences in the rates of coincidence detection — at sites of LTD (parallel-fiber and Purkinje cells) and LTP (mossy fibers and interpositus nucleus) — that could occur as a function of lowered or raised firing thresholds, respectively.

The implications of this work are threefold. First, we conducted this study to provide support for tDCS as a viable method for modulating specific cerebellar functional outcomes. Previous studies have provided mixed findings; thus, replication is essential particularly due to the novelty of this burgeoning field of neurostimulation, implications for clinical intervention research, and national calls for replicating research in the field [37]. Second, we conducted this work to provide currently unknown details regarding polarity- and intensity-specific effects of tDCS on cerebellar timing processes. Third, this study aimed to provide additional, human-based evidence demonstrating the cerebellum's role in associative learning processes, such as timing.

Material and Methods

Participants

The Indiana University Human Subjects Institutional Review Board approved all study procedures. Participants were recruited from the Indiana University Department of Psychological and Brain Sciences undergraduate subject pool. After providing written informed consent, 102 healthy participants completed the dEBC tDCS paradigm (74 females, 28 males; mean age=18.9 years [standard deviation=1.2 years]; Table 1). Participants received either active tDCS stimulation (n=20 anodal 1.5mA, n=22 cathodal 1.5mA, n=19 anodal 2mA, n=21 cathodal 2mA), or sham stimulation (n=20). All participants were free of any severe neurological disorders that may impact cerebellar integrity as assessed by the Abnormal Involuntary Movement Scale (AIMS), International Cooperative Ataxia Rating Scale (ICARS), and Neurological Soft Signs (NSS). Participants completed a self-report questionnaire to screen for personal history of mental and physical health as well as family mental health history. Additional exclusion criteria included severe psychopathology, seizure activity, pacemakers, serious head injuries that resulted in loss of consciousness for more than five minutes, and prior exposure to tDCS in the previous three months.

Transcranial Direct Current Stimulation (tDCS)

The neuroConn DC Stimulator Plus (model number: 0021; serial number: 2122, Ilmenau, Germany) was used to deliver sham, anodal, or cathodal stimulation during the conditioning task. Both participant and researcher were blind to the stimulation conditions, which were

randomly assigned via codes generated by the Stimulator study mode. tDCS was delivered through two humidified sponge electrodes (each 5cm×7cm, surface area 35 cm^2) soaked in 0.9% saline solution. To administer anodal stimulation, the anode was placed over the left cerebellar region (3 cm lateral to the inion [30] and ipsilateral to the conditioning eye), and the cathode was placed over the ipsilateral buccinator muscle. The opposite configuration was used to administer cathodal stimulation. The current was delivered at 1.5mA or 2mA depending on the participant group with a ramp-up/ramp-down time of 30s. Stimulation began at the termination of the first conditioning trial block in the acquisition phase and lasted for 20 minutes, terminating before the first trial of the extinction phase. Stimulation was only administered during the acquisition phase as the effects of stimulation on the acquisition and timing of the conditioned response were the focus of the present study. Moreover, the first conditioning block was conducted without stimulation to determine any potential baseline group differences that might skew the interpretation of stimulation effects. In the sham condition, the stimulation was ramped-up over 30s and immediately ramped-down over 30s at the termination of the first trial block in order to mimic the tingling sensation experienced in the active stimulation conditions. Immediately following tDCS administration, participants were asked to rate the intensity to which they felt the sensations of tingling, itching, burning, pain, discomfort, headache, nausea, fatigue, and alertness during stimulation [38, 39] on a scale of none, mild, moderate, strong, or severe. One participant did not complete the questionnaire.

Delay Eyeblink Conditioning (dEBC)

All participants completed a 40-minute single cue-tone delay eyeblink conditioning paradigm consisting of 108 trials that were administered with a jittered inter-trial interval (ITI) ranging 10–20s [40]. At the onset, eight unconditioned stimulus (US) alone trials were administered. The conditioned response (CR) acquisition phase followed, in which 10 blocks of 10 trials were presented. Each block contained one conditioned stimulus (CS) alone trial and nine (CS-US) paired trials. The CS alone trial was randomly presented once during the last five trials within each block. During the CS-US trials, a 400ms, 1000Hz (80dB SPL) tone was delivered co-terminating with a 50ms air puff to the inner canthus of the left eye. To maintain wakefulness and engagement in the task throughout the experiment, neutral images from the International Affective Picture System (IAPS [41]) were presented between each trial, and participants were asked to rate the pleasantness of the pictures on a scale of 1–10 using a button response pad. In addition, participants were observed on a monitor by the researchers in order to be certain that their eyes remained open and they were attentive to the task.

Electromyography (EMG) was utilized to record the eyeblinks. A pair of bipolar EMG electrodes (8mm Ag/Ag-Cl; Model TD-23; MedAssociated, St. Albans, VT) was placed on the orbicularis palpebrarum muscle below the left eye and a ground electrode was placed on the forehead. A pair of eyeglass rims with copper tubing (1/16-inch diameter) fastened 1cm away from the inner canthus of the left eye was used to deliver the air puff (US; 10psi at source, 50ms duration). The copper tubing was connected via a plastic tube (120 inch) to a regulator receiving medical grade air. The tone (CS; 1000Hz, 80dB SPL) was presented through foam ear inserts (E-A-RLINK, Aearo Company Auditory Systems, Indianapolis,

IN). All electrode impedances were maintained below 10kΩ. EMG data were continuously recorded at 2.5kHz with a Sensorium EPA-6 bioamplifier (high-pass filter=1Hz, 12dB/octave; low-pass filter=300Hz, eighth order elliptic; gain=5,000) and were acquired using Neuroscan software (Version 4.2; El Paso, TX).

Data Analysis

Data were analyzed using a well-established processing stream (c.f., [40]). Individual paired CS-US trials were epoched (1086ms) from the continuous data file beginning 500ms prior to onset of the CS. A high-pass filter (10Hz, 6dB/octave) was applied to the data before being rectified. Data were entered into Datamunch analysis software [42] for further analysis. Alpha responses, which are reflexive, non-associative orienting EMG responses to the tone CS, were assessed between 25ms and 100ms after the CS. On a subject-by-subject basis, responses were recorded as blinks if the amplitude exceeded five standard deviations above the baseline (baseline window for each trial=125ms prior to CS onset). CRs were recorded if the blink occurred between 100ms and 350ms after CS onset, which corresponded to a period beginning 250ms before the onset of the US. Trials in which spontaneous blinks occurred within a window from 75ms prior to and 25ms following CS onset were excluded from further analysis. Percent CRs and peak and onset latencies, determined as the time from the beginning of the CS to the peak or onset of the CR, were calculated.

The initial 8 US alone trials were epoched beginning 250ms before the onset of the US to 300ms after the onset of the US. Then, the data were high-pass filtered at 28Hz, rectified, and low-pass filtered at 10Hz. The data were then baseline corrected to the pre-stimulus period and peak amplitude values were extracted with a peak detection window of 50 to 250ms after stimulus onset.

In SPSS (v.26), a 10-by-5 repeated measures ANOVA was utilized to calculate the percent CRs as the dependent variable across the 10 blocks of the early and late conditioning (within subjects) and across the five stimulation groups (between subjects). This analysis was also done for CR onset and peak latencies as dependent variables. Level of significance was set at $p<0.05$ and a Greenhouse-Geisser adjustment was applied in instances of sphericity violation.

Physical sensations experienced by subjects during tDCS administration were analyzed to determine effects of stimulation (i.e., polarity and intensity) using the nonparametric Kruskal-Wallis test. In the case of a significant Kruskal-Wallis test, pairwise comparisons were subsequently performed using a Bonferroni correction for multiple comparisons.

Results

Participants did not differ on age, sex, or ethnicity (Table 1) across treatment groups (i.e., polarity, intensity).

CR Acquisition

Overall, learning as measured by increased percent CRs over time was observed across the 10 CS-US paired trial blocks across all active and sham stimulation groups for both tDCS

intensities (Figure 1A). Specifically, using a repeated-measures ANOVA, a significant block effect ($F(6.744, 654.133) = 38.89$; $p<0.001$) was found, indicating that percent CRs increased across trials. There was no main effect of stimulation group ($F(4,97)=0.833$; $p=0.5$) and no significant block by stimulation group interaction effect ($F(26.975,654.133)=1.183$; $p=0.24$).

Exploratory post-hoc analyses investigating only early acquisition (i.e., blocks 1–5) was performed due to an unexpected decrease in percent CRs following block 5 in the sham group. A significant block effect was found ($F(3.56, 345.285) = 35.99$; $p<0.001$). However, no main effect of stimulation group ($F(4, 97)=1.148$; $p=0.339$) or significant block by stimulation group interaction effect ($F(14.239, 345.285)=0.713$; $p=0.764$) was observed. In addition, a one-way ANOVA testing response amplitude in the first 8 US alone trials between the three groups revealed no significant group differences ($F(4,101)=0.68$; $p=0.608$).

CR Timing

Active tDCS stimulation affected CR peak and onset latencies (Figure 1B and 1C, respectively). There were significant main effects of stimulation ($F(4,97)=9.535$; $p<0.001$) and block ($F(6.361,617.035)=6.387$; $p<0.001$) and a significant block by stimulation interaction effect ($F(25.448, 617.035)=1.709$; $p=0.017$) on CR peak latency (Table 2). Bonferroni-corrected post-hoc t-tests indicated that active stimulation groups of both strengths and polarities had increased peak latency compared to the sham condition, with initial group differences starting at block 3.

For CR onset latency, a significant main effect of stimulation ($F(4,97)=8.51$; $p<0.001$) and block by stimulation interaction effect ($F(25.521, 618.875)=1.966$; $p=0.003$) were observed. No significant block effect was found ($F(6.38, 618.875)=0.967$; $p=0.45$). Bonferroni-corrected post-hoc t-tests indicated that active stimulation groups of both strengths and polarities had increased onset latency compared to the sham condition (Table 2), with initial group differences starting at block 3.

Physical Sensations Experienced During Stimulation

There was a significant difference between treatment groups in the reporting of a tingling sensation during tDCS administration ($H(4)=16.413$, $p=0.003$). Subsequent pairwise comparisons revealed a significant difference ($p=0.001$) in reports of greater tingling in the 1.5mA anodal group (mean rank = 66.11) than the sham group (mean rank=31.05) and a trend toward significance ($p=0.053$) in reports of greater tingling in the 2mA anodal group (mean rank=56.00) than the sham group. There were no significant differences between stimulation groups in reporting of itching, burning, pain, discomfort, headache, nausea, fatigue, or alertness.

Discussion

The aim of this study was to manipulate the timing of the conditioned responses (CRs) in delay eyeblink conditioning (dEBC) using three experimental stimulation conditions of transcranial direct current stimulation (tDCS; anodal, cathodal, and sham) and two tDCS intensities (1.5mA and 2mA) in order to study the cerebellum's role in timing processes. As

predicted, tDCS impacted the timing of the conditioned response in cerebellar-dependent dEBC. However, this effect was not shown to be polarity-dependent, contrary to predictions, as both polarities and intensities of stimulation altered CR timing processes.

Even though all groups exhibited conditioned learning over time, no polarity- or intensity-specific differences in CR acquisition were observed in the active stimulation groups. This result differs with the Zuchowski and colleagues [34] study, in which researchers found that cathodal stimulation significantly decreased percent CRs by 12.6±17.2% compared to the sham condition and anodal stimulation significantly increased percent CRs by 73.4±25.2% as compared to sham. However, the difference between the current study's findings and Zuchowski and colleagues [34] is likely a matter of sample size with the current study being more robustly powered to eliminate Type-I errors. Accordingly, similar to the current study, a follow-up study with larger sample size [32] by the same group found no significant differences in mean percent CRs between stimulation conditions. Additionally, an independent study [33] found that tDCS modulation of CR acquisition may be mediated by possession of the BDNF Val66Met polymorphism. Thus, differences in the effect of tDCS on CR acquisition may be affected in part by genetic variations among subjects.

Although blocks 1–5 showed strong conditioning rates across all stimulation groups, there was an unexpected decline in conditioning during blocks 5–10 in the sham group. The fall-off at block 5 in the sham group may have been the result of fatigue during the task. In contrast, participants receiving stimulation might have stayed alert for longer due to the physical sensations of tDCS. This is supported by the significant increase in reports of a tingling sensation in participants receiving 1.5mA anodal stimulation, and trending significance for the 2mA group, compared to sham group participants.

In the current study, both the anodal and cathodal conditions, at both intensities, significantly differed from the sham condition in the CR peak and onset latencies. Participants in the sham condition exhibited shorter onset and peak latencies relative to the active stimulation groups. However, no polarity-dependent differences were found between the anodal and cathodal groups. Both anodal and cathodal conditions showed significantly longer peak and onset latencies, shifting the CR closer to the US onset. This behavior is more adaptive, as it would result in maximal eyelid closer as close to the CS as possible, thereby avoiding the negative consequences of a puff to the open and vulnerable eye. There is generally a learning curve in healthy adults in which peak and onset latency become longer as learning progresses, such that the adaptive CR eyeblink occurs progressively closer to the US air puff [40, 43, 44]. Notably, in cannabis users, schizophrenia-spectrum and bipolar disorders, and Autism Spectrum Disorders CR latency has been found to be shorter and therefore can be viewed as less adaptive [25, 26, 43, 45, 46].

However, findings from the current study contrast with Zuchowski and colleagues [34], wherein CR onset latencies for the anodal condition became shorter across blocks, shifting them closer to the CS onset. The group's follow-up study, in which they reported no differences in conditioned responses across stimulation, did not find any differences in timing latencies [32]. Again, underpowered samples (10 per group in the previous studies) may not reliably detect these subtle timing patterns. Taken together, participants in the sham

condition of the present study appeared to learn the timing in this task as expected. However, both active conditions of tDCS seem to be facilitating the ability to learn the timing associated with the CS and US, as indicated by timing onset and peak occurring closer to the US onset. Cerebellar tDCS stimulation on EBC extinction was not evaluated in this study because previous literature has yielded no significant effects [32, 34, 47], notably including the replication study by Beyer and colleagues. Furthermore, such effects would be difficult to interpret because tDCS was not administered during extinction, and the current literature on tDCS after-effects is highly variable.

Rampersad and colleagues [30] found that tDCS administered to the cerebellum is most optimal in a human model for stimulating intended brain regions as compared to other regions of the cerebrum. This is due to the cerebellum's proximity to the skull. Nonetheless, there are a number of mechanistic aspects of tDCS that are not fully understood. Specifically, recent studies suggest that the effects of direct current stimulation are highly dependent on neuronal orientation in the target area [35, 48], which has important implications for cerebellar tDCS given the complexity of cerebellar architecture. As demonstrated by the current study, tDCS can impact cerebellar function, evidenced by facilitation of timing in both anodal and cathodal groups. It is not yet clear how deep into the cerebellum the current can reach, and it is also unknown if stimulation is acting on the entire cerebellum or just the cerebellar cortex, which is nearer to the scalp.

Recent advances in realistic modeling of electric fields produced by tDCS [49] may allow for more precise targeting of cerebellar structures in future studies [50]. Moreover, it is not known which cell types in the cerebellum are most affected (e.g., Purkinje cells, climbing fibers, Mossy fibers, parallel fibers, basket cells, etc.). It seems unlikely that tDCS can fully penetrate as deep as the cerebellar nuclei, specifically the IP nucleus, because the literature would suggest that learning of the conditioned response would then be significantly inhibited, which was not the case even with the cathodal group. However, it is likely that the cerebellar cortex was impacted by stimulation since both cathodal and anodal stimulation improved timing of the CR peak and onset.

In line with this theory, a review of dEBC [17] indicated that large lesions over the cerebellar cortex that did not impact the IP nucleus did not abolish conditioned response behavior but did interfere with learning the timing. Furthermore, in animal studies, lesions to cerebellar cortical lobule HVI, Crus I and II, and the ansiform lobe impacted the magnitude and timing of the conditioned response in the afflicted animals [17]. Additionally, the literature suggests that anodal stimulation increases neuronal activity by lowering the firing threshold, which seems to be the case for timing in this study. However, since there is a limited understanding of the complete mechanisms by which tDCS functions, other explanations are possible. For example, on a cellular level, tDCS might be impacting specific processes such as protein synthesis, gene expression, and channel activation [51].

Stimulation administered to other areas of the brain including the sensorimotor cortex and other parts of the cerebrum has altered neurotransmission of GABA, glutamate, brain-derived neurotrophic factor (BDNF), Tyrosine kinase B, and myoinositol; it is possible that such altered neurotransmission may also be occurring during cerebellar tDCS [51]. It has

been shown that tDCS can cause other physiological changes such as modulation of various metabolite levels in targeted cerebral structures [52], though it is not known if similar changes occur during cerebellar tDCS. Recently, van der Vliet and colleagues [33] found that susceptibility to tDCS modulation of CR acquisition in dEBC differed between carriers and non-carriers of the BDNF Val66Met polymorphism. In all, this provides evidence that tDCS is causing micro-scale changes to neurotransmission, but whether the micro-scale changes lead to functional changes is dependent on other factors. The presence of other factors, such as genetic polymorphisms, were not evaluated in the current study and may have increased error within stimulation groups, obscuring a CR acquisition effect.

Other limitations of this study include relatively small subgroup sample sizes (n=19 to 22 per treatment group), and a sample that was restricted in age range and predominantly female, which could limit the generalizability of the findings. Future studies should focus on using a larger and more diverse sample as well as utilizing this paradigm with clinical populations. tDCS is already being used in clinical studies to decrease clinical symptoms following stroke [51], major depressive disorder [53], drug addiction, acute and chronic pain, as well as other neurological and psychiatric disorders [31], such as the hallucinations observed in schizophrenia [54]. The current study suggests that tDCS may also be useful for those experiencing cerebellar deficits such as cerebellar degeneration, lesion patients, and/or individuals diagnosed with schizophrenia, wherein deficits in dEBC may be ameliorated by active tDCS stimulation. Lastly, replication is essential, especially for this technology for which there is still much to be understood regarding the mechanisms and effects of tDCS, as there are inconsistencies in results across studies.

In all, the results of this study indicate that cerebellar-dependent timing processes can be enhanced by tDCS in healthy individuals. Moreover, these effects do not appear to be polarity-specific, which may be in part due to the varied alignment of cells within the cerebellum, nor are they dependent of stimulation intensity, at least between 1.5 and 2.0 mA. The findings provide further support for cerebellar contributions to human eyeblink conditioning and for exploring therapeutic tDCS interventions for cerebellar dysfunction.

Acknowledgements

We wish to thank Karen Lorite-Gomez for her assistance with data collection.

Funding

This work was supported by the National Institutes of Health (grant number T32 MH103213 to WPH, ABM, and NBL; R01 MH074983 to WPH; R21 MH091774 to BFO; Indiana Clinical and Translational Sciences Institute award TL1 TR001107 and UL1 TR001108 to ABM); National Science Foundation (Graduate Research Fellowship Program Award 1342962 to NBL); and the Brain and Behavior Research Foundation (NARSAD Young Investigator Award to ARB).

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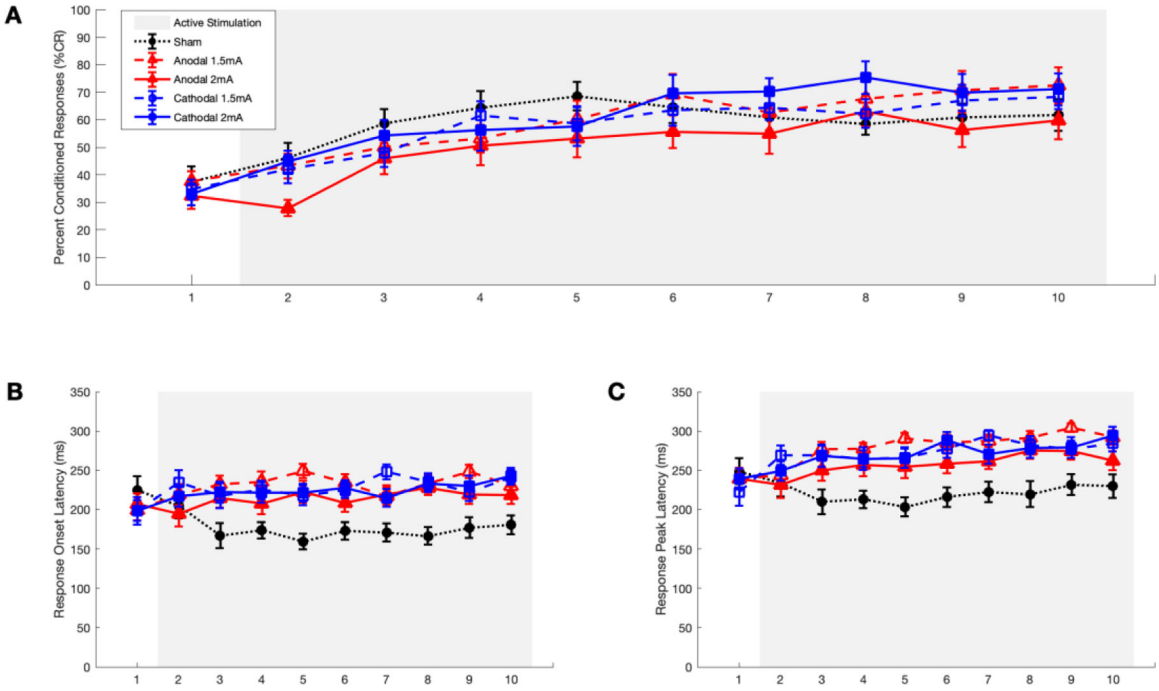

Figure 1.

Mean ± SE across blocks (x-axis) for delay eyeblink conditioning variables of interest. Gray shading denotes tDCS administration. (**A**) Percent CRs across blocks for each treatment group. While all groups showed evidence of learning, the groups did not differ significantly in conditioned response acquisition. (**B**) Conditioned response onset latency across blocks. All active stimulation groups exhibited significantly slower conditioned response onset latency compared to the sham condition, indicating more adaptively timed responses. (**C**) Conditioned response peak latency across blocks. All active stimulation groups exhibited significantly later conditioned response peak latency compared to the sham condition, indicating more adaptively timed responses.

Table 1.

Participant demographics. Values for sex and ethnicity reflect frequency; values for age reflect mean and standard deviation. F-statistic is from a one-way ANOVA. C, Caucasian; AA, African American; AS, Asian; H, Hispanic/Latino; O, Other.

Intensity		**1.5mA**		**2mA**			
Polarity	Sham (N=20)	Anodal (N=20)	Cathodal (N=22)	Anodal (N=19)	Cathodal (N=21)	χ^2 or F	p-value
Sex (M/F)	6/14	5/15	6/16	5/14	6/15	0.151	0.997
Age (years)	19.0 (1.3)	18.9 (0.9)	18.9 (1.4)	19.0 (1.7)	18.7 (0.8)	0.250	0.909
Ethnicity (C/AA/AS/H/O)	16/0/2/1/1	17/1/1/0/1	16/0/2/3/1	13/0/3/1/2	14/0/4/1/2	16.975	0.655

Table 2.

Post hoc tests for main effect of stimulation groups for timing parameters. Significant differences were found between sham (control group) and all four stimulation groups; Significance set at $p<0.05$ with Bonferroni correction for multiple comparisons.

	Stimulation Group (I)	Stimulation Group (J)	Mean Difference (I-J)	Std. Error	*p*-value
Peak Latency	Sham	Anodal 1.5mA	−56.13	10.19	<0.001
		Cathodal 1.5mA	−47.92	9.96	<0.001
		Cathodal 2mA	−46.94	10.07	<0.001
		Anodal 2mA	−33.62	10.32	0.016
Onset Latency	Sham	Anodal 1.5mA	−50.13	9.94	<0.001
		Cathodal 1.5mA	−46.93	9.71	<0.001
		Cathodal 2mA	−43.29	9.82	<0.001
		Anodal 2mA	−34.32	10.07	0.01