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Modulation of Multiunit Spike Activity by Transcranial AC Stimulation (tACS) in the Rat Cerebellar Cortex

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Abstract

Transcranial electrical stimulation (tES) techniques have garnered significant interest due to their noninvasiveness and potential to offer a treatment option in a wide variety of brain disorders. Among several modulation techniques, transcranial alternating current stimulation (tACS) is favored for its ability to entrain the neural oscillations. The cerebellum is one of the targeted sites because of its involvement in motor and cognitive functions. However, animal studies are lacking in the literature looking into the mechanism of action in cerebellar tACS. In this study, we used a rat model and monitored the activity of the cerebellar cortex, which sculpts the cerebellar output by adjusting the firing rate and timing of the neurons in the deep cerebellar nuclei (DCN). For neural recording, a tungsten electrode was inserted into the cerebellar cortex through a craniotomy hole located over the right paramedian lobule (PML). A helical Ag/AgCl wire electrode was placed atop the skull near the caudal edge to inject a 1 Hz biphasic sinusoidal current. Our results showed that the multiunit activity (MUA) of the cerebellar cortex was strongly modulated by tACS. The negative phase of the electric current enhanced the neural firing rate while the positive phase suppressed the activity. Furthermore, the spike rate showed modulation by the instantaneous strength of the injected current within the sinusoidal cycle. This warrants research to further look into the mechanism of tACS acting on the cerebellar cortex at the cellular level.

I. INTRODUCTION

Non-invasive brain stimulation techniques target neuronal structures and modulate their activity. Among these methods, transcranial electrical stimulations (tES), in particular transcranial direct current stimulation (tDCS) and alternating current stimulation (tACS), are reporting encouraging outcomes in clinical research [1, 2]. TDCS utilizes direct currents while tACs injects biphasic currents reversing the electron stream periodically. Both tDCS and tACS use weak currents and expectedly cause subthreshold modulation at the cellular level. Although the underlying mechanism of these methods still remains as an active area of research, it appears that, in addition to shifting the resting membrane potential, the applied electric field can also manipulate the neurotransmitter concentration in the microenvironment [3, 4].

Brain oscillations carry important information for cognitive functions [5] and aberration of these oscillations causes various mental disorders [6]. Modulation of these rhythmic activities offers treatment options to those who suffer from mental difficulties such as depression [7] and attention deficit [8]. Several studies have shown that the alternating current stimulation is able to synchronize intrinsic neural activities and entrain the endogenous oscillations. Frochlich *et al.* demonstrated that directing the weak sinusoidal current to a cortical slice preparation was able to provoke neural spikes and ultimately modify the multiunit activity [9]. Zaehle *et al.* also showed that tACS enhances the alpha oscillation and its effect continues after the end of stimulation [10]. Several other studies also reported the long-lasting effect of tACS [11, 12] thus suggesting neuroplasticity. The change on the spike activity and phase shift led by tACS are considered to be the mechanism behind the plasticity observed [11, 13].

Unlike the cerebrum, there has not been extensive research looking at the effect of tACS on the cerebellar circuitry. Traditionally, the cerebellum was thought to be involved only in the motor function and the fine movements. However, studies over the past decades revealed the crucial role of the cerebellum on cognitive functions such as language processing [14] and visuospatial attention [15]. The cerebellar cortex receives inputs from different parts of the brain and the periphery via the mossy and climbing fiber afferents. In response, the Purkinje cells (PCs) generate the sole output from the cerebellar cortex in the form of simple and complex spike activities and shape the cerebellar output by regulating the timing and firing rate of the cells in the deep cerebellar nuclei (DCN). Complex spike activity of the PCs causes the low frequency oscillations (1–4 Hz) [16], while simple spikes can lead to oscillations at very high frequencies (160–260 Hz) [17]. In this regard, entrainment of the oscillations in the cerebellar cortex can allow manipulation of the cerebellar output on the DCN. In his seminal work, Chan *et al.* applied a 1Hz sinusoidal electric field to the isolated turtle cerebella with varying intensity levels and demonstrated the modulation of the PCs and Stellate cells [18]. Naro *et al.* also applied tACS over the right cerebellar hemisphere at 10, 50, 300Hz and measured their effects on the motor evoked potentials (MEP). Their findings show that while 50 Hz tACS increases the MEP, 10 and 300 Hz-tACS cause mild or no effect on the MEP [19]. To understand the mechanism behind the cerebellar tACS, further *in vivo* experiments are necessary to examine the response of the local cerebellar circuits. The main goal of this study was to probe the multiunit activity in the cerebellar cortex during tACS application in a rat model. Our results demonstrate that the PC activity can be modulated quite strongly with transcranial application of alternating currents.

II. METHODS

A. Surgery

Two Sprague Dawley rats (320–350 g) were used in this study. All procedures were approved and performed in accordance to the guidelines of the Institutional Animal Care and Use Committee (IACUC), Rutgers University, Newark, NJ. Animals were anesthetized in an induction chamber with 5% isoflurane and then moved to the stereotaxic frame. Isoflurane level was lowered to 1–3% and adjusted according to the depth of anesthesia. Before starting the neural recordings, anesthesia regime was switched to the ketamine/xylazine (80mg/kg

and 12mg/kg, IP) mixture and additional doses of ketamine were injected as needed during the course of surgery. The blood-oxygen level and heart rate were monitored with a pulse oximeter. Body temperature was measured by a rectal probe and regulated at 36°C with a heating pad under the animal.

B. Neural Recording

The skin and the muscle tissue over the right cerebellum were removed to perform a craniotomy of ~2×2mm. The dura was punctured with a sharp 31g needle and cut with micro-scissors. A layer of 125µm thick silicone (PDMS) with a small window at the center was laid out over the cortex as an artificial dura to prevent dehydration and motion caused by breathing and cardiac pulsation. A tungsten electrode (5 MΩ, TM33B05H, World Precision Inst.) was slowly inserted into the superficial layers of the cortex with the help of a 10µm step-size micromanipulator. A Ag/AgCl wire was attached over the skull as a recording reference electrode. Neural activity was simultaneously monitored on the oscilloscope and listened through an audio amplifier with a speaker while searching for the Purkinje cell layer with the tungsten electrode inside the cerebellar cortex.

C. Electrical Stimulation

The skin over the right caudal edge of the skull was removed and connective tissue was scraped off to place the stimulation electrode. A Ag/AgCl wire was wrapped around a 1mm rod to form a helix and used as a stimulation electrode. The helical electrode was placed juxtaposed to the caudal edge of the lambdoid suture which was a few mm above the recording electrode. The center of the helical electrode was filled with a conductive gel to maximize the contact area with the skull and obtain a uniform current distribution. Another Ag/AgCl wire was inserted into the ipsilateral shoulder as a return electrode for stimulation current. For tACS application, the AC waveform was generated through the computer controlled current generator (Model 2200, A-M Systems). Each recording episode consisted of 5 s of no stimulation followed by 10 sinusoidal cycles of AC at 1 Hz. Upon the completion of the first set of neural recording, tungsten electrode was repositioned to obtain another set of recording. This process was repeated two times in rat1 and three times in rat 2. Amplitudes of the applied currents were varied between 0.5–1.0 mA.

D. Data Collection and Analysis

The neural recordings were performed in a large Faraday cage through a physiological amplifier (Model 1700, modified for high-input impedance, A-M Systems, WA) with filter setting at 100Hz–10kHz. Recorded signals were sampled at 100kHz through a National Instruments data acquisition board (PCI 6071) controlled via MATLAB software. The recordings were divided into 3 sections; the baseline activity, and the activities during positive and negative phases of the AC. The baseline activity was used as a control recording and compared with the stimulated intervals. The number of spikes during the baseline recording was counted with a peak detection algorithm and the obtained value was divided by the duration to compute the mean firing rate. The algorithm used a threshold value determined based on the signal to noise ratio to detect peaks. Signals during AC stimulation were divided into 10 cycles and then the firing rates during the positive and negative phases of each cycle were calculated separately before taking their mean.

III. RESULTS

Multiunit Spike Activity During tACS

The cerebellar MUA was effectively modulated by tACS. First 5 s in Fig. 2 demonstrates the baseline activity and the following 10 s during the 1 Hz tACS. Spike activity increases during the negative phase of the sinusoidal stimulus with maximum values coinciding with the peak. On the contrary, activity decreases when the cranial electrode is positive and almost ceases around the peak. Figure 3 shows the raster plot for all 5 recordings in two different rats. All recordings have similar firing patterns except Trial 4 where the effect of stimulus is reversed during positive and negative cycles. Fig. 4 shows the mean firing rates and standard deviations across the ten periods of the sinusoidal stimulation.

IV. DISCUSSION

The cerebellar tACS has been investigated in human subjects, and several potential benefits have been reported. However, animal studies investigating the direct impact of tACS on neurological activity are lacking in the literature. The present study investigated tACS effects on the multiunit spike activity of the cerebellar cortex in an *in-vivo* rat model. The neural activity recorded with tungsten electrodes resembled the activity of PCs as suggested by their high firing rates. An effort was not made to identify them as such by searching for complex spikes. Although a single cell was mostly dominant in the recorded signals, we treated the signals as multi-unit spike activity since we cannot eliminate the possibility of recording more than one cell at a time. In five different trials in two rats, there was not a clear correlation with the tACS amplitude and the depth of modulation. There are two potential explanations for this. Due to local inhomogeneities in the electrical conductivity of neural tissue, the electric field experienced by nearby cells may not be the same, as we reported in a separate study [20, 21]. Therefore, neurons in each recording might be exposed to different intensity levels of the field and responded differently.

It has been validated by several animal and computational studies that proximal regions of the neuronal structures to the anodic electrode are hyperpolarized while the distal compartments are depolarized under extracellular electric fields. In this respect, previously Bindman *et al.* in the cerebral cortex [22], and lately Bikson *et al.* in the hippocampus [23] showed that anodal stimulation enhances the neural activity whereas cathodal stimulation suppresses it. Chan *et al.* also applied 1 Hz sinusoidal currents to cerebellar slices and reported similar effects related to orientation [18]. In the present work, we observed a decline in the activity during the anodic cycle and an increase during the cathodic phase, except in one case (Trial 4). The discrepancy is likely to emerge from the orientation of the neurons with respect to the direction of the applied electric field. The cerebellum has a highly folded structure. We recorded the neural activity from the PC layers at different depths and did not pay attention to the orientation of the neurons relative to the field. Another explanation could be that the stimulation affects the interneurons that are part of the same PC circuitry in the cerebellar cortex, although the modulatory effect is expected to be less due to their relatively smaller sizes than the PCs, and as a result the net effect varies in different cortical regions. Therefore, in order to probe the response of individual cell types, the electric stimulation needs to be delivered locally to the region of interest. Chan *et al.* also

reported that the neurons on the cerebellar cortex, PCs and Stellate cells, are synchronized with 1 Hz AC *in vitro* which was corroborated by our findings in an *in vivo* preparation under anesthesia.

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REFERENCES

1. Schutter DJLG and Wischniewski M, A meta-analytic study of exogenous oscillatory electric potentials in neuroenhancement. *Neuropsychologia*, 2016 86: p. 110–118. [PubMed: 27085766]
2. Dedoncker J, et al., A Systematic Review and Meta-Analysis of the Effects of Transcranial Direct Current Stimulation (tDCS) Over the Dorsolateral Prefrontal Cortex in Healthy and Neuropsychiatric Samples: Influence of Stimulation Parameters. *Brain Stimulation*, 2016 9(4): p. 501–517. [PubMed: 27160468]
3. Nitsche MA and Paulus W, Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *The Journal of Physiology*, 2000 527(3): p. 633–639. [PubMed: 10990547]
4. Stagg CJ, et al., Polarity-Sensitive Modulation of Cortical Neurotransmitters by Transcranial Stimulation. *The Journal of Neuroscience*, 2009 29(16): p. 5202–5206. [PubMed: 19386916]
5. Knyazev GG, Motivation, emotion, and their inhibitory control mirrored in brain oscillations. *Neuroscience & Biobehavioral Reviews*, 2007 31(3): p. 377–395. [PubMed: 17145079]
6. Buzsáki G and Draguhn A, Neuronal Oscillations in Cortical Networks. *Science*, 2004 304(5679): p. 1926–1929. [PubMed: 15218136]
7. Fitzgerald PJ and Watson BO, Gamma oscillations as a biomarker for major depression: an emerging topic. *Translational psychiatry*, 2018 8(1): p. 177–177. [PubMed: 30181587]
8. Lenartowicz A, et al., Aberrant Modulation of Brain Oscillatory Activity and Attentional Impairment in Attention-Deficit/Hyperactivity Disorder. *Biological psychiatry. Cognitive neuroscience and neuroimaging*, 2018 3(1): p. 19–29. [PubMed: 29397074]
9. Fröhlich F and McCormick DA, Endogenous electric fields may guide neocortical network activity. *Neuron*, 2010 67(1): p. 129–143. [PubMed: 20624597]
10. Zaehle T, Rach S, and Herrmann CS, Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PloS one*, 2010 5(11): p. e13766–e13766. [PubMed: 21072168]
11. Vossen A, Gross J, and Thut G, Alpha Power Increase After Transcranial Alternating Current Stimulation at Alpha Frequency (α -tACS) Reflects Plastic Changes Rather Than Entrainment. *Brain Stimulation*, 2015 8(3): p. 499–508. [PubMed: 25648377]
12. Kasten FH, Dowsett J, and Herrmann CS, Sustained Aftereffect of α -tACS Lasts Up to 70 min after Stimulation. *Frontiers in Human Neuroscience*, 2016 10(245).
13. Herrmann CS, et al., Transcranial alternating current stimulation: a review of the underlying mechanisms and modulation of cognitive processes. *Frontiers in human neuroscience*, 2013 7: p. 279–279. [PubMed: 23785325]
14. Booth JR, et al., The role of the basal ganglia and cerebellum in language processing. *Brain research*, 2007 1133(1): p. 136–144. [PubMed: 17189619]
15. Yamaguchi S, Tsuchiya H, and Kobayashi S, Visuospatial Attention Shift and Motor Responses in Cerebellar Disorders. *Journal of Cognitive Neuroscience*, 1998 10(1): p. 95–107. [PubMed: 9526085]
16. De Zeeuw CI, Hoebeek FE, and Schonewille M, Causes and Consequences of Oscillations in the Cerebellar Cortex. *Neuron*, 2008 58(5): p. 655–658. [PubMed: 18549777]
17. de Solages C, et al., High-Frequency Organization and Synchrony of Activity in the Purkinje Cell Layer of the Cerebellum. *Neuron*, 2008 58(5): p. 775–788. [PubMed: 18549788]

18. Chan CY and Nicholson C, Modulation by applied electric fields of Purkinje and stellate cell activity in the isolated turtle cerebellum. *The Journal of physiology*, 1986 371: p. 89–114. [PubMed: 3701658]
19. Naro A, et al., Does Transcranial Alternating Current Stimulation Induce Cerebellum Plasticity? Feasibility, Safety and Efficacy of a Novel Electrophysiological Approach. *Brain Stimulation*, 2016 9(3): p. 388–395. [PubMed: 26946958]
20. Asan AS, Gok S, and Sahin M, Electrical fields induced inside the rat brain with skin, skull, and dural placements of the current injection electrode. *PLOS ONE*, 2019 14(1): p. e0203727. [PubMed: 30629578]
21. Asan AS, Gok S, and Sahin M. Electric Fields Induced By Transcutaneous And Intracranial Current Injections In The Rat Brain. in 2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). 2018.
22. Bindman LJ, Lippold OC, and Redfearn JW, THE ACTION OF BRIEF POLARIZING CURRENTS ON THE CEREBRAL CORTEX OF THE RAT (1) DURING CURRENT FLOW AND (2) IN THE PRODUCTION OF LONG-LASTING AFTEREFFECTS. *The Journal of physiology*, 1964 172(3): p. 369–382. [PubMed: 14199369]
23. Bikson M, et al., Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *The Journal of Physiology*, 2004 557(1): p. 175–190. [PubMed: 14978199]

Figure 1.

Recording and stimulation electrode placements. The helical Ag/AgCl stimulation electrode was positioned on the right caudal edge of the skull and the recording electrode was inserted into the PML cortex of the cerebellum. The inset shows the PML area on the lateral cerebellum.

Figure 2.

Multiunit spike recording (from Trial 5). The red sinusoidal signal represents the injected current as a function of time. Upper row shows a small episode of the recorded signal on a larger time scale.

Figure 3.

Raster plot of all 5 recordings (Trial 1–2) from rat1 (Trial 3–5) from rat2. The top row exemplifies the shape of the applied current and rows below show the spike timings in each recording.

Figure 4. Firing rates during the baseline activity and during the positive and negative phases of the AC stimulus. Errorbars are the standard deviation.