2021 43rd Annual International Conference of the
IEEE Engineering in Medicine & Biology Society (EMBC)
Oct 31 - Nov 4, 2021. Virtual Conference

ECoG Power Alterations Across Stages of Prolonged Transcorneal Electrical Stimulation in the Blind Mice *

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***Abstract*— Transcorneal electrical stimulation (TES) is a non-invasive approach for activating the retina and its downstream components through the application of electric current on the cornea. Although previous studies have demonstrated the clinical relevance of TES for modulating neurons with improvements in visual evoked potentials (VEPs) and electroretinograms (ERGs), there are still huge gaps in knowledge of its effect on the brain structures. To determine the short-term impact as well as the after-effects of TES on neural oscillatory power in retinal degeneration mice, we performed electrocorticography (ECoG) recording in the prefrontal and primary visual cortices at different stages of prolonged TES [transient stage, following prolonged stimulation (post-stimulation stage 1) and long after the end of the retinal stimulation (post-stimulation stage 2)]) under varying stimulation current amplitudes (400 μA, 500 μA and 600 μA). The results revealed asymmetric differences between short-term and long-term pTES under different stimulation current amplitudes. Specifically, in post-stimulation stage 1 we observed significant increase in ECoG power of theta, alpha and beta oscillations respectively compared with baseline pre-stimulation results. These effects were dependent on the stimulation current amplitude and stimulation stage. Transient TES was not sufficient to cause significant changes in the ECoG power of all accessed oscillations except in medium, high and ultra-gamma oscillations which significantly decreased in 400 μA and 500 μA stimulation groups respectively compared with pre-stimulation results. Regarding long-term stimulation, the increase in ECoG power of theta, alpha and beta oscillations observed in post-stimulation stage 1 was significantly maintained in post-stimulation stage 2.**

***Clinical Relevance*— These results could be of core importance for human TES protocols suggesting that following pTES and long after the end of the stimulation, TES current amplitudes could have relatively different impacts on the power/activity of cortical oscillations. For example, by increasing the activity of oscillations that have been reported to inhibit irrelevant neural processes and enable the brain to focus on more relevant neural processes thus, inducing better coordination in the cortex.**

I. INTRODUCTION

Blindness is a devastating condition that arises from several sources including retinal degeneration diseases such as retinitis pigmentosa (RP) and age-related macular degeneration (AMD). Globally, RP affects an estimated number of 1 in 4000 individuals worldwide [1,2] and AMD alone accounts for vision loss in more than 67 million elderlies of age 60 in the western world [3-4]. Morphometric studies have provided viable evidence that confirms the sparing of some retinal neurons in the face of wide spread retinal degeneration. In severe RP patients, 30% of retinal ganglion cells and 88% of other inner retinal neurons have been confirmed to survive degeneration [5-6]. Similarly, in AMD patients much higher survival rates (53–97%) of inner retinal neurons have also been reported [7].

As a consequence of the aforementioned observations and reports, researchers now target these surviving retinal neurons using various invasive and non-invasive electrical stimulation approaches in hopes of activating the retinal circuitry and restoring visual functionality. Transcorneal electrical stimulation (TES) is one of such approaches that has been severally used to non-invasively stimulate the retina and its downstream structures with documented improvements in electroretinograms (ERGs) [8] and visual evoked potentials (VEPs) [9]. Integral to the production of an effective approach for vision restoration is a clear understanding of brain oscillations which are fundamental drivers of electrical signals sent to neurons in the brain. Indeed, the activities of neural oscillations specifically during the resting-state are key factors that determine neural circuit development [10] as well as spatio-temporal organization of neural networks [11].

Electrocorticography (ECoG) has been widely dubbed as the gold standard for extracting cortical electrical activity and analyzing neural oscillations. Generally, brain oscillations are divided into five frequency bands ranging from slow high amplitude waves of delta (0-5 Hz), theta (5-10 Hz) and alpha (10-15 Hz), to fast low amplitude oscillations of beta (15-30 Hz) and gamma (> 30 Hz).

In the present study we set out to understand changes in brain oscillations that accompany electrical stimulation of the degenerated retina. On this basis we used ECoG to specifically monitor the alteration of neural oscillatory power in both prefrontal cortex and primary visual cortex during different stimulation stages and current amplitudes of prolonged transcorneal electrical stimulation (pTES).

*Research supported by the City University of Hong Kong under Projects CityU Project 7005452, the Research Grant Council of the Hong Kong Special Administrative (Grant No.: CityU 11208218, 11207419), and the Innovation and Technology Fund Guangdong-Hong Kong Technology Cooperation Funding Scheme (GHP/078/18GD).

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II. MATERIALS AND METHODS

A. Animal Preparation and Surgery

In order to mimic human retinal degeneration, we used rd10 mice as the animal model of RP. Rd10 mice has the same phosphodiesterase-6-β-mutation as the human RP and degeneration of rd10 mice starts at post-natal day (P18). All mice were housed in City University of Hong Kong Laboratory Animal Research Unit. In all experiments, all protocols of the present study were approved by the Animal Research Ethics Sub-committee at City University of Hong Kong and Department of Health, HKSAR. A total of 15 males and 15 female rdl0 mice of age P60 - P90 were used. For the surgical protocol, Ketamine-Xylazine anesthesia (Ketamine: 100 mg/kg, Xylazine: 10 mg/kg) was initially administered to all mice. Subsequently, a mixed concentration of Isoflurane (1.5%) and oxygen (0.4%) was added to maintain the sleep state of the mice during the entire surgery. Lubritha (Alfamedics, Hong Kong, HKSAR) eye gel was applied regularly to preserve eye moisture of the mice throughout the surgery. For craniotomy, the mice were head-fixed on the sterotaxis and their dura was exposed. Stainless bone-screw recording electrodes with shaft diameter of 2.4 mm (Decorah LLC, GEC, North America) were implanted over the left prefrontal cortices (AP: 2 mm, 1 mm from the midline) and primary visual cortex (AP: -3.5 mm, 2.5 mm from the midline). A reference screw electrode was positioned in the cerebellum. A heating pad (Model # TP702; Gaymar Industries, Inc., NY, USA) was used in keeping the mice body temperature at a constant value of 37°C while the heart rate was carefully monitored

B. Electrical Stimulation of the Retina

Transcorneal electrical stimulation (TES) was used to stimulate the mice retina. Briefly, isoflurane (1.5%) - oxygen (0.4%) mixture was used to anesthetize the mice then a silver wire electrode (impedance: 0.143 kΩ at 1 kHz) was placed over the right cornea. This setup was connected to STG4004 electrical stimulator (Multi Channel Systems, Baden-Württemberg, Germany) to generate and deliver a charge-balance biphasic square pulse to the mice eye. A reference needle electrode was positioned subcutaneously near the stimulated eye. TES was performed for a prolonged duration of five days at 30 min per day. Three stimulation groups (10 mice per group) were set up and each group was stimulated with various current amplitudes of 400 μA, 500 μA and 600 μA respectively at 10 Hz stimulation frequency and 2 ms/phase pulse width.

C. Recording of Resting State Electrocorticography

Resting state ECoG activity was recorded over the left prefrontal cortex and left primary visual cortices in awake rd10 mice. Recording was performed under ambient lighting conditions. An active signal transfer cable was connected to the implanted recording electrode. ECoG signal was acquired and amplified by the A-M Systems 3600 (A-M Systems, Washington, US) and CED Micro 1401-3 (Cambridge Electronic Design, UK). Briefly, the ECoG signal was filtered from 0.3 Hz to 300 Hz at a sampling rate of 5 kHz. Pre-stimulation baseline ECoG recording was first performed for three days, subsequently post-stimulation recording was performed at different stages as stated below.

Transient stage post-stimulation recording: This was performed on day 1 of the prolonged stimulation experiment.

Post-stimulation stage 1 recording: This was performed following the end of the five days prolonged stimulation.

Post-stimulation stage 2 recording: This was performed after one week following the end of post-stimulation stage 1.

D. Power Spectral Analysis

The recorded ECoG signals were down-sampled from 5 kHz to 1 kHz to save computational resources and time consumption for power spectrum analysis. An IIR notch filter was used to remove the 50-Hz power-line noise and its resultant harmonics. The absolute power was estimated using Welch's modified periodogram method, with a 2-sec epoch length and 50% overlap between contiguous epochs where each epoch was windowed with a Hamming window. The absolute power was assessed for each ECoG segment over all brain oscillation frequency bands (0.1–300 Hz). Across all stimulation groups and stimulation stages, the mean and SEM of absolute power for eight frequency bands [delta (δ; 0.5 - 5 Hz), theta (θ; 5-10 Hz), alpha (α; 10-15 Hz), beta (β; 15-30 Hz), low gamma (γ_1; 30-55 Hz), medium gamma (γ_2; 60-115 Hz), high gamma (or γ_3; 125 - 175 Hz), and ultra-gamma (γ_4; 185 - 300 Hz) was computed.

D. Statistical Analysis

Statistical analysis was performed with Origin (Pro) software (Version 2015, Origin Lab Corporation, Northampton, MA, United States). To test the statistical significance of the change in absolute power across the four stages of stimulation in each stimulation group, one-way ANOVA and Tukey's multi-comparison tests were carried out for the eight frequency bands. The adjusted P-values by Tukey's correction was *P < 0.025.

III. RESULTS

To index changes in the power spectrum of brain oscillations during different stages of pTES (transient stage, post-stimulation stage 1 and post-stimulation stage 2), resting-state ECoG signals were recorded from the left prefrontal cortex and left primary visual cortex of awake rd10 mice following electrical stimulation at various current amplitudes (400 μA, 500 μA and 600 μA). The results revealed that transient stage pTES yielded no significant changes in the ECoG power (p>0.025) across all stimulation groups and all other brain oscillations (Figures 1 and 2) except in gamma bands (Figure 2). With regards to this, transient pTES triggered significant decrease of medium gamma, high gamma and ultra-high gamma ECoG power respectively in both 400 μA and 500 μA stimulation groups compared to the pre-stimulation results (Figure 2). Following post-stimulation stage 1, all stimulation groups showed significant increase in theta and alpha ECoG power in compared to pre-stimulation stage (Figures 1 and 2). In all stimulation groups, this increase was found to be maintained long after the end of the stimulation process (i.e., in post-stimulation stage 2) (Figures 1 and 2). For changes in beta oscillations, we observed significant increase in ECoG power of beta oscillations in only 500 μA stimulation group. This change was again observed to be maintained in the post-stimulation stage 2 (Figures 1 and 2).

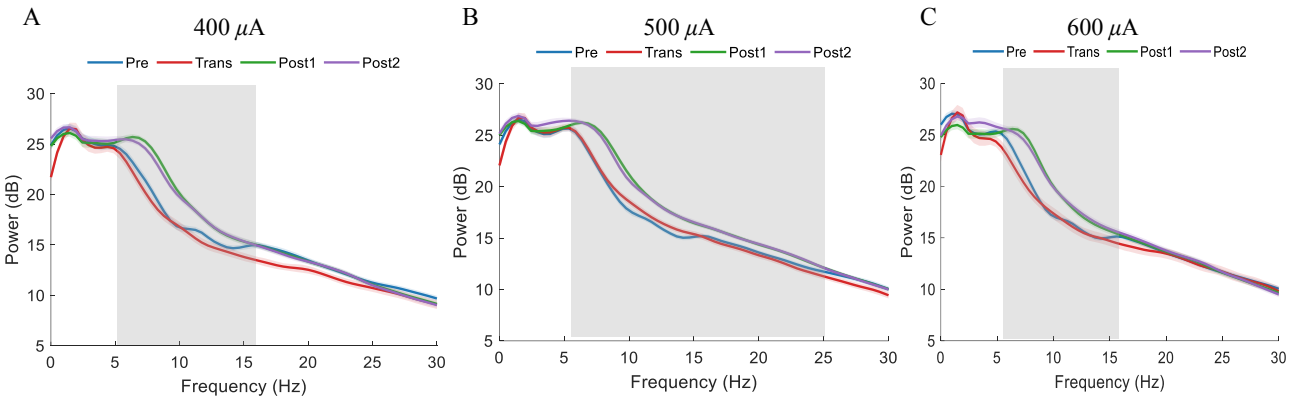

Figure 1. Spectral power profile of both prefrontal cortex and primary visual cortex at different stages of prolonged transcorneal electrical stimulation (pTES). Rd10 mice were stimulated at 400 μA (A), 500 μA (B) and 600 μA (C) current amplitudes. All stimulation groups showed significant increase in theta and alpha spectral power in post-stimulation stage 1 compared to pre-stimulation stage. 500 μA stimulation current caused a broader increase in spectral power of brain oscillations.

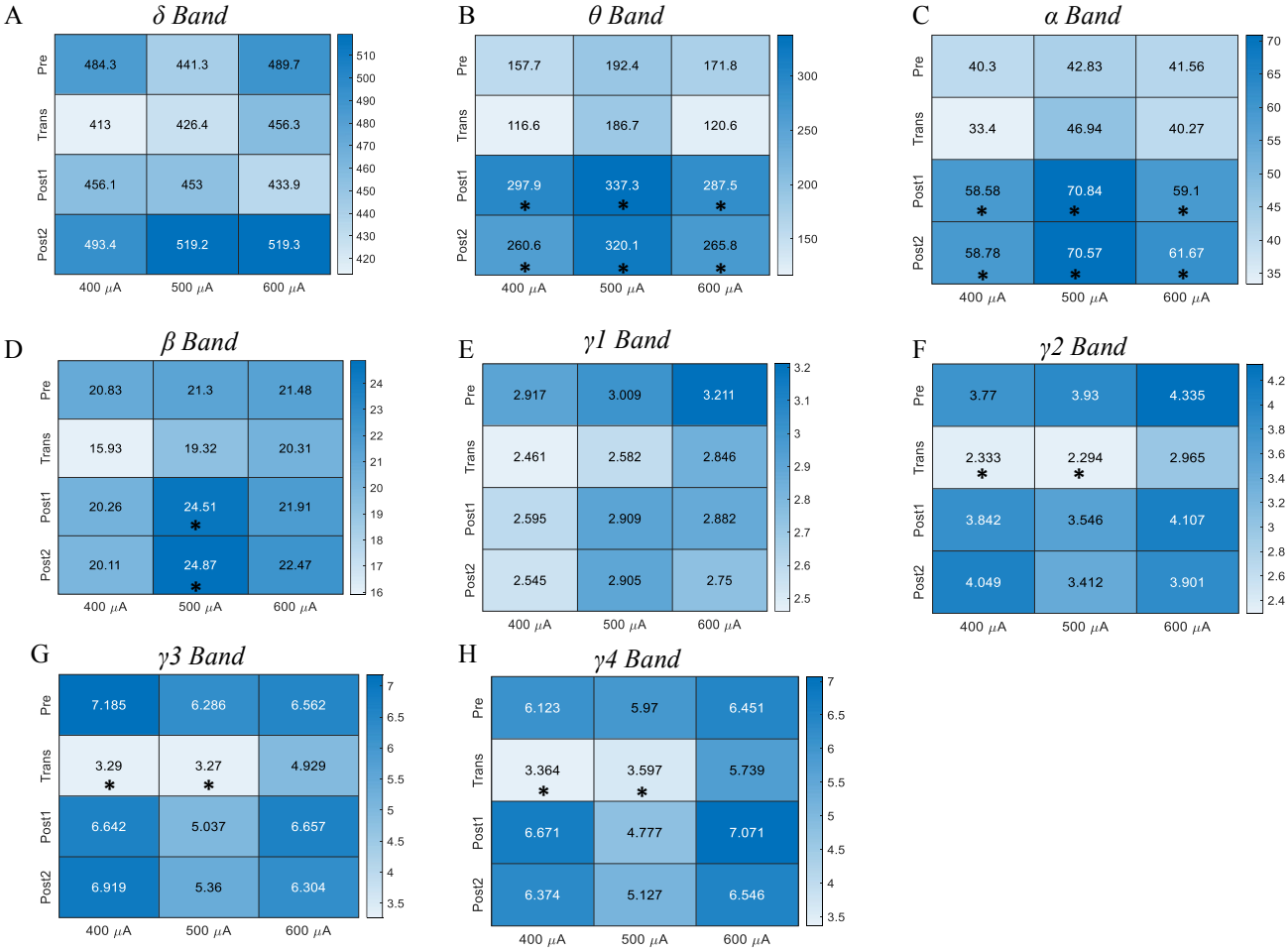

Figure 2. Heat map of ECoG absolute power at different stages pTES. The absolute power of 8 brain oscillatory bands (A-H) was averaged over the left prefrontal and left primary visual cortex of rd10 before (pre-stimulation stage) and following prolonged pTES (transient stage, post stimulation stage 1 and post-stimulation stage 2). We observed significant increase ($p<0.025$) in absolute power of theta, alpha and beta oscillations respectively and significant decrease ($p<0.025$) in ECoG power of gamma oscillations which were all dependent on the stimulation current amplitude and stimulation stage. * Denotes significant ($p<0.025$).

IV. DISCUSSION

The present electrophysiological results point to asymmetric differences between short-term and long-term effects observed after pTES under different stimulation current amplitudes. These results could be of core importance for human TES protocols suggesting that the TES current amplitudes could have relatively different impacts on the power/activity of cortical oscillations following pTES and long after the end of the stimulation process.

The main aim of the present study was to understand changes in brain oscillations that accompany electrical stimulation of the degenerated retina by directly quantifying the induced oscillatory power changes at spatially separated cortical regions of the brain. As expected, in both the left prefrontal cortex and the left primary visual cortex we observed significant increase in ECoG power of theta, alpha and beta oscillations respectively and significant decrease in ECoG power of gamma oscillations which were all dependent on the stimulation current amplitude and stimulation stage.

Increase in theta oscillations have been reported to be important for facilitating cognitive performance including working memory functions and visual attention [12]. On the other hand, beta oscillations have been demonstrated to track or register changes that occur before and after task execution in the cortex. Both theta and beta oscillations have been implicated in serving as indices for integration and consolidation of neural inputs [13]. In the present study, the increase and maintenance of theta and beta power during post-stimulation stage 1 and post-stimulation stage 2 may indicate increase in cognitive function of the blind rd10 mice as well as consolidation of relevant changes in cortical neural activity that occurred in both the left prefrontal cortex and the left primary visual cortex after pTES. In this regard, further investigations on the cognitive assessment after electrical stimulation is warranted.

Across all stimulation groups, the power of alpha oscillations was significantly increased in post-stimulation stage 1 and maintained in post-stimulation stage 2. Increased alpha activity has been severally demonstrated to have inhibitory effects in the neural activities of the brain. Specifically, alpha waves have been reported to drastically reduce irrelevant neural processing in the cortex thus enabling the brain to process more informative neural signals [14]. Neurodegeneration diseases including those affecting the auditory cortex, visual cortex and other deep brain structures have often be associated with spontaneous firing which has been suggested to represent desynchronization and idling of the neurons. Specifically, it has been demonstrated that retinal neurodegeneration SS3-ter rats exhibited greater spontaneous firing of the primary visual cortex neurons compared to the wild type controls [15]. Gamma oscillation have long been a marker of increased neural firing in various areas of the mammalian cortex [16]. While transient stimulation yielded no significant change in the power of the other oscillations across all stimulation groups, it was necessary for decreasing broad band gamma power (medium, high and ultra-gamma) in 400 μA and 500 μA stimulation groups respectively. Consequently, the significant decrease in gamma oscillations we observed in the transient state and the significant increase in alpha waves that appeared in both post-stimulation stage 1 and post-stimulation stage 2 could imply that pTES alters the activities of both alpha and gamma oscillations in reducing neuron idling and irrelevant spontaneous firing of cortical neurons.

Although the present study does not investigate visual restoration to the blind animals however, previous studies with rd animals have reported some improvements in functional and histological analysis of the retina [17] such as preserved ERGs and increased outer nuclear layer thickness respectively after TES.

V. CONCLUSION

In summary, the present electrophysiological study clearly shows differences in neural oscillatory power between short-term and long-term pTES across varying stimulation current amplitudes in blind rd10 mice. The complexity of the present reported effects of pTES on ECoG power provides a clear insight on how brain oscillations are altered after non-invasive electrical stimulation. Indeed, this is crucial to the production of an effective therapy for vision restoration by not only studying retinal neural responses to electrical stimulation, but also going beyond the eye and studying the inner workings of the brain.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. Yi Wang, Ms. Xin Li and Mr. Chung-Yuen Chan for their contributions to the experimental setup and experimental protocol.

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