Brain Stimulation 14 (2021) 771–779

Contents lists available at ScienceDirect

Brain Stimulation

journal homepage: http://www.journals.elsevier.com/brain-stimulation

Delta oscillation underlies the interictal spike changes after repeated transcranial direct current stimulation in a rat model of chronic seizures

Yi-Jen Wu [a, b, *], Miao-Er Chien [a], Chia-Chu Chiang [c], Ying-Zu Huang [d, e, f], Dominique M. Durand [c], Kuei-Sen Hsu [g, h, **]

[a] Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, Tainan 70457, Taiwan
[b] Department of Neurology, National Cheng Kung University Hospital, College of Medicine, National Cheng Kung University, Tainan 70403, Taiwan
[c] Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106, USA
[d] Department of Neurology and Neuroscience Research Center, Chang Gung Memorial Hospital at Linkou, Taoyuan City, Taiwan
[e] Medical School and Healthy Aging Research Center, College of Medicine, Chang Gung University, Taoyuan, Taiwan
[f] Institute of Cognitive Neuroscience, National Central University, Taoyuan, Taiwan
[g] Department of Pharmacology, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan
[h] Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan 70101, Taiwan

ARTICLE INFO

Article history:
Received 10 November 2020
Received in revised form
27 April 2021
Accepted 29 April 2021
Available online 11 May 2021

Keywords:
Seizure
Epilepsy
Electroencephalography (EEG)
Oscillation
Interictal spikes
Transcranial direct current stimulation (tDCS)

ABSTRACT

Background: Transcranial direct current stimulation (tDCS) provides a noninvasive polarity-specific constant current to treat epilepsy, through a mechanism possibly involving excitability modulation and neural oscillation.
Objective: To determine whether EEG oscillations underlie the interictal spike changes after tDCS in rats with chronic spontaneous seizures.
Methods: Rats with kainic acid-induced spontaneous seizures were subjected to cathodal tDCS or sham stimulation for 5 consecutive days. Video-EEG recordings were collected immediately pre- and post-stimulation and for the subsequent 2 weeks following stimulation. The acute pre-post stimulation and subacute follow-up changes of interictal spikes and EEG oscillations in tDCS-treated rats were compared with sham. Ictal EEG with seizure behaviors, hippocampal brain-derived neurotrophic factor (BDNF) protein expression, and mossy fiber sprouting were compared between tDCS and sham rats.
Results: Interictal spike counts were reduced immediately following tDCS with augmented delta and diminished beta and gamma oscillations compared with sham. Cathodal tDCS also enhanced delta oscillations in normal rats. However, increased numbers of interictal spikes with a decrease of delta and theta oscillations were observed in tDCS-treated rats compared with sham during the following 2 weeks after stimulation. Resuming tDCS suppressed the increase of interictal spike activity. In tDCS rats, hippocampal BDNF protein expression was decreased while mossy fiber sprouting did not change compared with sham.
Conclusions: The inverse relationship between the changes of delta oscillation and interictal spikes during tDCS on and off stimulation periods indicates that an enhanced endogenous delta oscillation underlies the tDCS inhibitory effect on epileptic excitability.

Introduction

Seizure is a transiently abnormal symptom of excessive synchronized neuronal discharge in the brain [1]. Epilepsy is a brain disorder where the neural network has an enduring tendency to spontaneously generate recurrent seizures [2]. Current antiepileptic drugs (AEDs) can only effectively control seizures in up

* Corresponding author. Institute of Clinical Medicine, College of Medicine, National Cheng Kung University, No. 35, Xiaodong Rd., Tainan City 70457, Taiwan.
** Corresponding author. Department of Pharmacology, College of Medicine, National Cheng Kung University, No. 1, University Rd., Tainan City 70101, Taiwan.
E-mail addresses: wuyj@mail.ncku.edu.tw (Y.-J. Wu), richard@mail.ncku.edu.tw (K.-S. Hsu).

https://doi.org/10.1016/j.brs.2021.04.025

to two-thirds of patients with epilepsy [3]. For patients with drug-resistant epilepsy, surgery to remove or disconnect epileptogenic regions provides a post-operative seizure free rate around 30–80% [4]. Neuromodulation therapeutics such as deep brain stimulation and vagus nerve stimulation are currently FDA-approved, while others like transcranial magnetic stimulation and transcranial direct current stimulation (tDCS) are undergoing clinical trials for patients with refractory epilepsy [5,6]. tDCS is a non-invasive, safe, and easy-to-use stimulation modality to treat seizures in patients with epilepsy [7]. tDCS can modulate cortical excitability by polarity-dependent direct current stimulation, whereby anodal tDCS (a-tDCS) and cathodal tDCS (c-tDCS) provide facilitatory and inhibitory effects, respectively [8,9]. In general, c-tDCS can decrease cortical excitability and cause long-term depression (LTD)-like plasticity. However, c-tDCS can also generate non-linear effects with excitability enhancement and long-term potentiation (LTP)-like plasticity [10,11]. In addition, long-lasting effects and neuroplasticity changes following repeated tDCS have also been reported in animal models of disease such as neuropathic pain and cognitive impairment [12,13]. Repeated tDCS could be a treatment for neurological disorders by modulating cortical excitability and related dysfunctions, such as seizure and epilepsy. Indeed, some clinical studies showed that c-tDCS reduced seizure frequency in patients with epilepsy [14–19]. Nevertheless, the interictal epileptiform discharge (IED) changes following tDCS exhibit large inter-individual variability. An IED is a transient epileptic potential without behavioral seizures that reflects a brief event of hyper-excitability on EEG. Though IEDs do not fully represent the general background activity and synchronicity of the neural network, they indicate a clear abnormality of network hyper-excitability. Network excitability is associated with changes in EEG oscillations, but little is known about the relationship between changes in EEG oscillation and IED in epilepsy animal models treated with repeated tDCS. Understanding how tDCS modulates oscillations and affects neuronal excitability in the epileptic brain is important to determine tDCS' mechanism of action. Ictal EEG oscillations have been shown to be altered in tDCS-treated status epilepticus (SE) rats [20]. Low frequency stimulation was reported to reduce seizures by deep brain stimulation [21] and optogenetic stimulation [22]. Although direct current (DC) is not like those modalities with variable stimulation frequencies, it is interesting to determine whether tDCS changes the excitability in an epileptic brain by altering its endogenous rhythms. Here, we hypothesize that repeated c-tDCS can decrease excitability, as measured by the change of interictal spikes, through modulating neural oscillations in a rodent model of epilepsy. The study investigates the changes of interictal spikes and EEG power spectral density (PSD) during the immediate pre- and post-stimulation periods, as well as the follow-up periods following withdrawal of repeated tDCS compared with sham stimulation in a chronic rat model of kainic acid (KA)-induced spontaneous seizures.

Methods

Chronic rat model of KA-induced spontaneous seizures

The chronic rat model of spontaneous seizures following KA-induced SE was adopted in this study [20,23,24] (Supplementary Methods). Four weeks after KA-induced SE, chronic spontaneous seizures and interictal spikes appeared [25], and the rats were subjected to the tDCS experiment. Ninety-one Sprague–Dawley male rats (6 weeks old, 200–270 g, KA-tDCS = 25, KA-sham = 21, normal-tDCS = 23, normal-sham = 19, KA = 3) were used. All experimental procedures were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of National Cheng Kung University.

Depth EEG implantation and tDCS assembly

The surgery was performed in the third week after KA induction and animals had 1 week for recovery before the experiments. Animals were placed on the stereotaxic apparatus under anesthesia to implant a plastic cannula (1 cm-height, 1 mm-inner radius) fixed on the skull, with the cannula center located 3 mm posterior to the Bregma on the central sagittal fissure above the dorsal hippocampus. The cannula was filled with 0.9% normal saline to serve as a plugin site for the c-tDCS electrode pin [20,26,27]. An EKG electrode (30×22 mm^2 pad, Ambu/NF50K) was placed at the dorsal shoulder to serve as a-tDCS electrode and fixed with conductive gel during stimulation [13]. A DC stimulator (DC-Stimulator Plus, NeuroConn) was used to generate 1 mA cathodal DC over the defined skull area above the dorsal hippocampus with 10 s-ramp up and 10 s-ramp down. Five bipolar depth EEG needles (50 μm-diameter PFA-coated stainless-steel wire, A-M Systems, WA, USA) were inserted into bilateral CA1 (AP: 5 mm, ML: ± 3.5 mm, DV: 3 mm) and aligned with three on the right CA1 and two on the left CA1 with 50 μm distance in-between. The c-tDCS electrode and EEG needles were fixed onto the skull using acrylic dental cement (Sigma).

tDCS stimulation and EEG recording protocol

EEG signals on the day before stimulation were recorded for 2 h as baseline (D0). Rats were subjected to tDCS (1 mA, 30 min per day) or sham (1 mA, 30 s per day) for five consecutive days. This setting has been shown to alleviate seizure severity in SE rats [20] and chosen for our study. Experiments were performed under identical isoflurane gas anesthesia to reduce stress and ensure stability during stimulation, and all procedures were identical in both groups. Video-EEG recording was performed in awake freely moving rats for 1 h before and after stimulation each day (D1–5) to compare the acute pre-post stimulation changes in KA rats. Identical stimulation procedures (1 mA, 30 min for c-tDCS; 30 s for sham) were performed in normal rats with pre- and post-stimulation (stim) recordings for 20 min, respectively. In contrast to the stimulation groups, the EEGs of age- and time-matched KA rats were obtained for 5 days. The recording was continuous on every other day for 6 h per day in the following period after stimulation in KA rats (D8–19). Sham or tDCS was resumed for another 5 days (D22–26) after the follow-up period when the immediate pre- and post-stim EEGs were recorded. The procedure of the second stimulation period was analogous to the first. All animals received stimulation and EEG recording at identical periods of a day. EEGs were collected with a gain of 800 and 0.8 Hz to 7 kHz band pass filter at a 2 kHz sampling rate. Signals were collected using a neural recording system (5 channels Tethered Record System, Triangle BioSystems International) and processed through the MP150 acquisition system (BIOPAC Systems Inc.). All EEG signals were analyzed off-line.

Interictal spike detection and EEG oscillation analysis

Spike detection

Interictal spikes were automatically detected using the following criteria: (1) peak amplitude > 10 times the baseline amplitude and > 1 mV, and (2) width at the half maximum of the peak < 200 ms with an inter-peak-interval to the prior detected spike > 70 ms [20]. The baseline amplitude for each rat was calculated by averaging the absolute amplitude values from three segments of 2-min EEGs starting at 0, 20, and 40 min from the 1-h

EEG. We calculated the pre-post stim spike change ratio by subtracting pre-stim from post-stim spike counts and then divided by the pre-stim spike number, 1 h for each pre- and post-stim recording period. An identical method was applied to calculate the follow-up to pre-stim spike change ratio whereby D0 EEG was used as pre-stim baseline.

Oscillations

Post-tDCS EEGs (D1–5) were analyzed using PSD and compared with those of sham in KA-treated rats and normal rats, respectively. PSDs of the follow-up EEG (D8–19) between tDCS and sham in KA rats were also compared. The baseline-normalized post-stim EEG was processed using Welch's method for PSD, using every 2000-point segment with 50% overlapping (MatLab built-in function, pwelch). The difference between tDCS and sham was displayed on the time frequency spectrogram over the 120 s sampled and summed from the 28–30 and 58–60 min, and 1200 s from 0–20, 20–40, and 40–60 min of the post-stim 1 h EEG from D1–5. Pre- and post-stim power spectra were integrated, calculated into the pre-post ratio (post-stim divided by pre-stim) for delta, theta, alpha, beta and gamma bands and compared between tDCS and sham-treated KA rats. The correlation between pre-post stim change ratios of interictal spike and delta power was examined.

BDNF immunoassay and mossy fiber immunofluorescent staining

At the end of the 2-week follow-up recording period, randomly selected KA-induced animals from the tDCS and sham groups were sacrificed to obtain hippocampal tissues for brain derived neurotrophic factor (BDNF) analysis and mossy fiber immunofluorescent staining. BDNF protein levels were measured using a conventional ChemiKine BDNF Sandwich ELISA kit (Chemicon/Millipore, Billerica, MA) according to the manufacturer's protocol. The hippocampus was sliced for Zinc transporter 3 (ZnT3) mossy fiber staining with 40 μm thickness with one out of six slices being selected for staining. Coronal brain sections were incubated with primary antibodies against ZnT3 followed by secondary antibodies. Fluorescence microscopic images were obtained using a confocal laser scanning microscope (FV1000, Leica, Germany), and imported into TissueQuest software version 4.0 for ZnT3 intensity quantitative analysis. The ZnT3 intensity of the granular cell layer and molecular layer over the dentate gyrus was automatically counted for each slice. The intensity in each rat was then divided by the mean of all sham-treated rats to obtain the ZnT3 intensity ratio. (Supplementary Methods).

Statistical analysis

The results are presented as median ± interquartile range (IQR). All statistical analyses were performed using the Prism 6 software package. The differences of interictal spikes, frequency specific oscillations, BDNF and ZnT3 staining between tDCS and sham were compared using the non-parametric Mann–Whitney *U* test for non-normal data distributions. PSD comparison between groups was analyzed using repeated measure two-way ANOVA. The level of significance was set at $p < 0.05$.

Results

Interictal spike activity decreased immediately after c-tDCS

We first examined whether the interictal spike activity in a chronic spontaneous seizure rat model was affected by tDCS. Before stimulation, the baseline interictal spike frequency obtained in the fourth week following KA induction (D0) was not significantly different between the tDCS and sham groups (median of interictal spikes per hour, tDCS 27.64 vs. sham 28.62, Mann–Whitney $U = 185.0$, $p = 0.916$). The pre-post spike number change ratios were lower in tDCS rats compared with sham ($p = 0.023$; Fig. 1A). The pre-post spike number change ratios were then chronologically displayed on each stimulation day and compared between tDCS and sham. The pre-post spike number change ratios of the tDCS group decreased with less variability than sham, when a significant reduction in tDCS group was observed during D3 stimulation ($p = 0.033$; Fig. 1B). A decrease in interictal spikes was observed when comparing pre- and immediate post-stim EEGs from a representative tDCS-treated rat (Fig. 1C) versus a sham-treated rat (Fig. 1D). Neither tissue injury nor cell count differences between groups were observed after five stimulation days (Supplementary Fig. 1).

Delta oscillation was enhanced immediately after repeated c-tDCS

Since decreased interictal spike frequencies were observed immediately after tDCS, we further tested the hypothesis that the change in local field oscillations induced by tDCS was correlated with the interictal spike reduction. Two-way repeated measure ANOVA showed that tDCS was a significant factor contributing to PSD changes in KA rats (tDCS, $n = 25$, vs. sham, $n = 21$ rats, $p = 0.0369$ for right CA1, $p = 0.0021$ for left CA1, one electrode). Increased delta power in tDCS-treated rats versus sham was consistently observed from EEG sampled from two electrodes at the left CA1, three electrodes at the right CA1 and all five electrodes (Fig. 2A). Logarithmic PSD showed similar results as raw data analyses (Supplementary Fig. 2). Delta oscillation was significantly higher in tDCS-treated rats compared to sham-stimulation or KA induction only. To determine whether the increased delta oscillation is generated by c-tDCS, we examined the PSD of post-stim EEG comparing tDCS and sham in normal rats. tDCS remained a significant factor affecting EEG oscillation in normal rats ($p < 0.0001$, Fig. 2B), with delta oscillations being significantly increased in tDCS compared to sham-treated normal rats. We further examined pre-post tDCS power ratio changes in KA rats for each specific frequency band over five stimulation days compared with sham (Fig. 2D–H). High frequency gamma ($p < 0.0001$) and beta oscillations ($p = 0.0142$) were significantly reduced in tDCS-treated rats. In contrast, the low frequency delta oscillation power ($p = 0.0158$) significantly increased following repeated c-tDCS. The enhanced delta oscillation in tDCS-treated rats was clearly visible on the time frequency spectrogram following summation of the power ratio difference of tDCS and sham over each frequency across time (Fig. 2C and Supplementary Fig. 3). The negative correlation between the pre-post stimulation change ratios of interictal spikes and delta power (Spearman $r = -0.1670$, $p = 0.0337$), which was stronger in tDCS than sham-treated rats (Fig. 2I), indicates the association between the decrease of interictal spikes and increased delta power following stimulation.

Increased interictal spikes and decreased low-frequency oscillation in the follow-up period after tDCS withdrawal

The interictal spikes recorded during the 2-week period following stimulation were analyzed to investigate whether the inhibitory effect on interictal spikes would be affected by tDCS withdrawal in KA rats. When pooling data sampled from every other day among D8–19, we found that the follow-up to pre-stim spike change ratio significantly increased in the tDCS group ($n = 20$) compared with sham group ($n = 14$ rats, Mann–Whitney *U* test, $p < 0.0001$, Fig. 3A). Follow-up to pre-stim interictal spike change ratios increased in tDCS-treated rats compared to sham

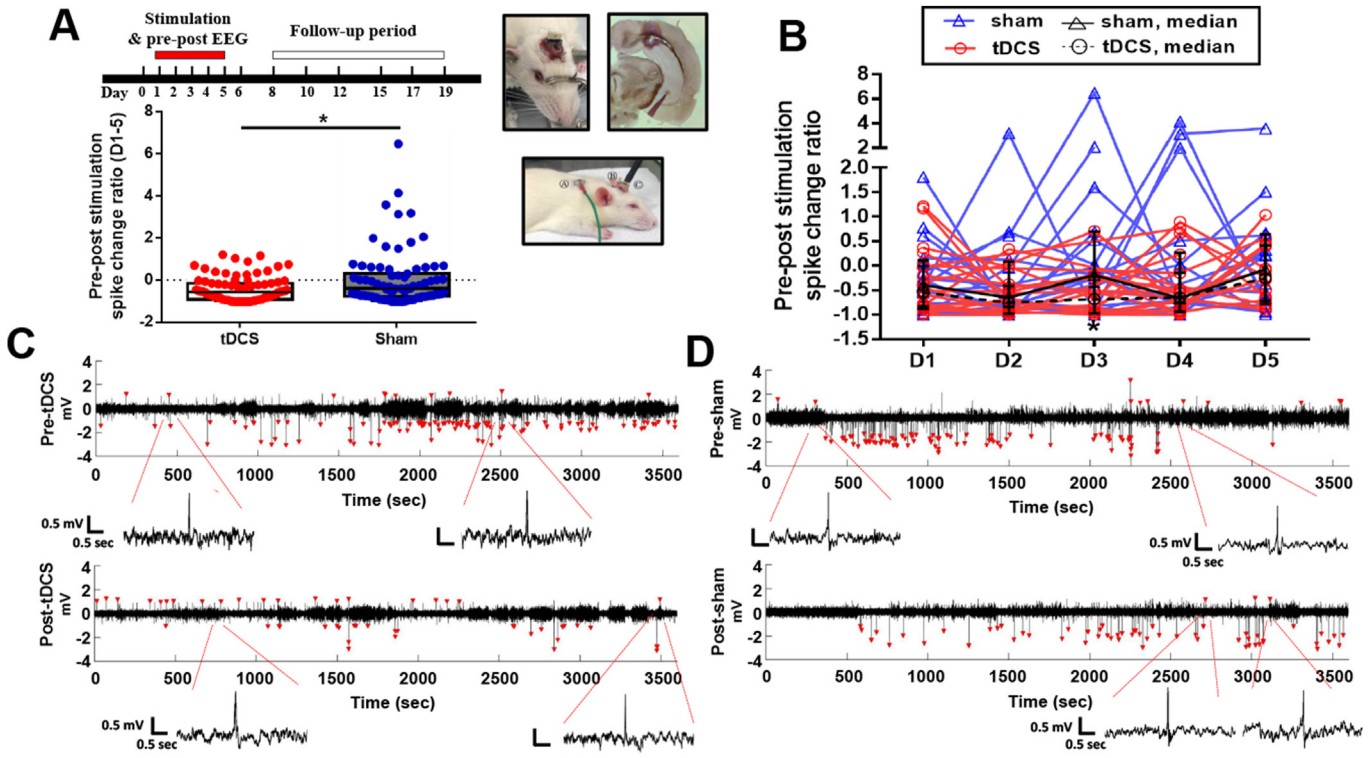

Fig. 1. Interictal spike activity decreased immediately after c-tDCS. A, Experimental time course (upper left panel). Red bar indicates pre- and post-stimulation EEG sampling period. Assembly of EEG electrode and c-tDCS electrode plugin site (upper middle panel). Electroablation of the depth EEG needle insertion site (upper right panel). Stimulation setup, Ⓐ anodal electrode, Ⓑ EEG assembly, Ⓒ cathodal electrode (lower right panel). Post-to pre-stimulation spike change ratio over the 5 days of stimulation in tDCS treated rats compared with sham (lower left panel, tDCS $n = 25$ rats, median $= -0.580$, sham $n = 21$ rats, median $= -0.394$, Mann–Whitney U test, $p = 0.023$). Dark lines indicate median and boxes interquartile range (IQR). **B**, Pre-post interictal spike change ratio on each stimulation day in tDCS and sham-treated rats. A significant reduction on D3 between tDCS and sham (median, tDCS $= -0.679$ vs. sham $= -0.188$, Mann–Whitney $U = 51.5$, $p = 0.033$). Pre-post spike daily change ratio for each animal shown in blue or red traces for sham and tDCS, respectively. Black traces with triangles and circles represent the median with IQR from sham and tDCS rats, respectively. **C**, Representative pre- and post-stimulation interictal spikes in a tDCS treated rat. **D**, Representative pre- and post-stimulation interictal spikes in a sham treated rat. *$p < 0.05$.

animals particularly on D12 and D15 (Fig. 3B). The PSD revealed a distinct difference between tDCS and sham-treated rats (tDCS vs. sham, $p < 0.0001$, two-way ANOVA). *Post-hoc* analysis showed that PSDs of tDCS rats significantly decreased from ≧ 1 Hz to < 8 Hz compared to sham (Fig. 3C). Isolated interictal spikes were sporadically scattered in representative traces of tDCS and sham rats (Fig. 3D) but not corresponding to delta-to-theta PSD changes. To test whether the increase of interictal spikes is a rebound response following tDCS withdrawal and can again be suppressed by another course of repeated tDCS, we recorded the EEG from the first stimulation period (D1–5), the follow-up (D6–21), and the second stimulation period (D22–26). In tDCS-treated rats, post-stim interictal spikes indeed decreased compared with the pre-stim during the first tDCS course. The frequency of interictal spikes increased in the follow-up period and was suppressed again by the subsequent tDCS session (Fig. 3E, left upper panel). Neither immediate post-stim suppression nor following-up spike rebound was observed in sham-treated rats (Fig. 3E, right upper panel). Interictal spikes in the follow-up period significantly increased compared to post-tDCS spikes of the first and second stimulation phases while no significant changes occurred in sham-treated rats ($n = 3$ in both groups, Fig. 3E, lower panel).

Ictal discharges in the follow-up period after repeated tDCS

Although no seizure was observed during the pre- and post-stim periods in tDCS and sham rats, we analyzed tDCS effects on ictal EEG and behavior in follow-up periods. Seven seizure events with simultaneous ictal EEG were recorded from tDCS and sham-treated rats: one “freezing” seizure, one stage 4 seizure (Racine scale) in tDCS rats and one stage 3, three stage 4, and one stage 5 seizures in sham rats (Supplementary Fig. 4). The seizures lasted for 1–2 min and subsided spontaneously. Quasi-rhythmic periodic discharges of isolated spikes were recorded in a tDCS rat with freezing behavior (Fig. 4A). Ictal EEG of a stage 4 seizure with high-amplitude polyspikes, followed by rhythmic spike-and-wave complexes (~1.5–2 Hz) and then regression (Fig. 4B) was recorded in another tDCS rat while presenting forelimb continuous clonus and rearing with lordotic standing posture. Ictal EEG recorded in one sham-treated rat during stage 4 and 5 seizures showed similar patterns with a burst of high-frequency dense polyspikes for around 15 s and returning to interictal state (Fig. 4C and D). In contrast to the sustained polyspikes observed in sham rats, low-frequency ictal spikes or spike-and-wave complex were commonly observed in tDCS rats.

Hippocampal mossy fiber sprouting and BDNF expression following repeated tDCS

Hippocampal mossy fiber sprouting and BDNF expression are reported as chronological consequences reflecting tDCS effects on severe seizures in a rat model of SE [20]. To investigate whether tDCS effects on our model of chronic spontaneous seizure can be reflected by mossy fiber sprouting and BDNF expression, we analyzed both in the hippocampi in tDCS and sham conditions. There was no significant difference on ZnT3 stained mossy fibers in the granular cell layer and molecular layer of the dentate gyrus

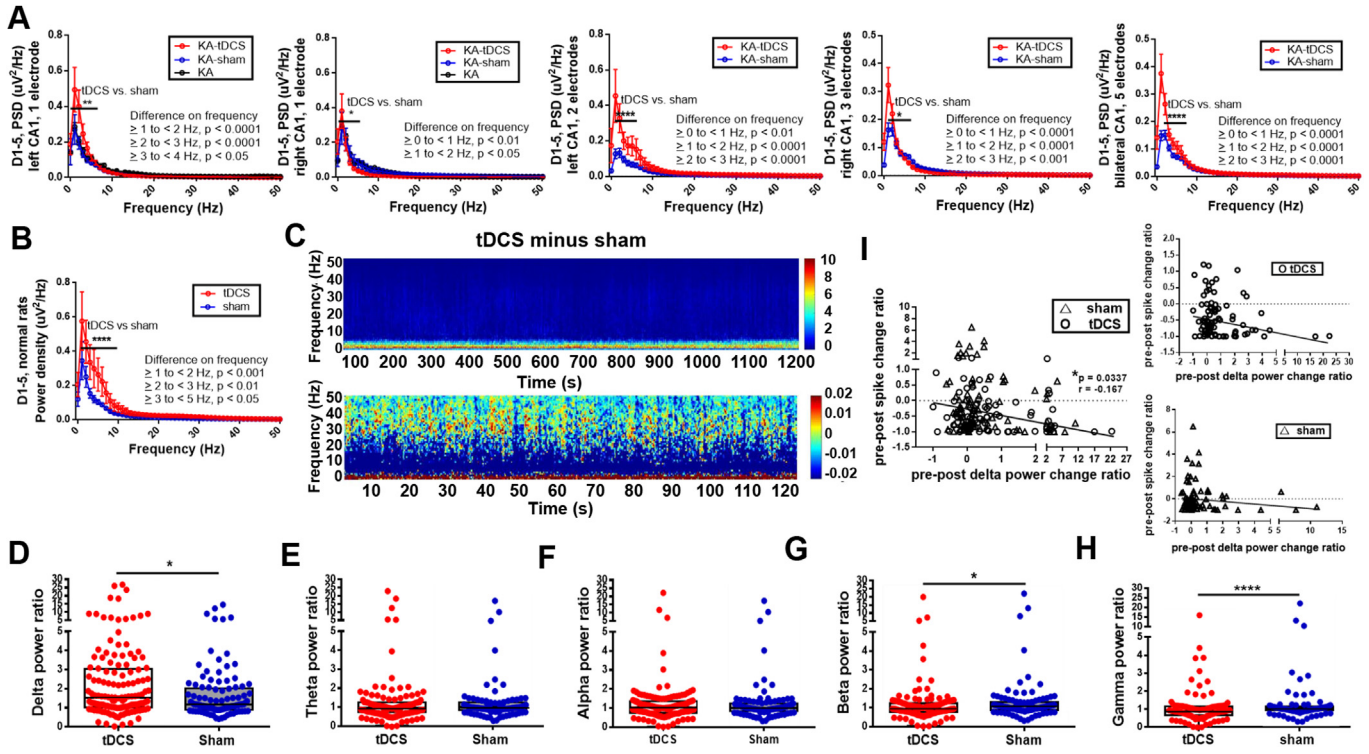

Fig. 2. Delta oscillation was enhanced immediately after repeated c-tDCS. A, Post-tDCS PSD in KA rats over the 5 stimulation days compared with sham stimulation (first panel, left CA1-1 electrode, two-way repeated measure ANOVA, tDCS vs. sham, $p = 0.0021$, frequency $p < 0.0001$, interaction $p < 0.0001$, *post-hoc* Bonferroni's test, tDCS vs. sham, ≧ 1 to < 2 Hz, $p < 0.0001$, ≧ 2 to < 3 Hz, $p < 0.0001$, and ≧ 3 to < 4 Hz, $p = 0.0153$; second panel, right CA1-1 electrode, tDCS vs. sham, $p = 0.0369$, and frequency $p < 0.0001$, *post-hoc* test, tDCS vs. sham, ≧ 0 to < 1 Hz, $p = 0.0071$ and ≧ 1 to < 2 Hz, $p = 0.0159$; third panel, left CA1-2 electrodes, tDCS vs. sham, $p < 0.0001$, frequency $p < 0.0001$, interaction $p < 0.0001$, *post-hoc* test, tDCS vs. sham, ≧ 0 to < 1 Hz, $p = 0.0027$, ≧ 1 to < 2 Hz, $p < 0.0001$, and ≧ 2 to < 3 Hz, $p < 0.0001$; fourth panel, right CA1-3 electrodes, tDCS vs. sham, $p = 0.0466$, frequency $p < 0.0001$, interaction $p < 0.0001$, *post-hoc* test, tDCS vs. sham, ≧ 0 to < 1 Hz, $p < 0.0001$, ≧ 1 to < 2 Hz, $p < 0.0001$, and ≧ 2 to < 3 Hz, $p = 0.0005$; fifth panel, bilateral CA1-5 electrodes, tDCS vs. sham, $p < 0.0001$, frequency $p < 0.0001$, interaction $p < 0.0001$, *post-hoc* test, tDCS vs. sham, ≧ 0 to < 1 Hz, $p < 0.0001$, ≧ 1 to < 2 Hz, $p < 0.0001$, and ≧ 2 to < 3 Hz, $p < 0.0001$). PSD of age- and time-matched KA rats indicated by black line. **B**, Post-tDCS PSD of normal rats over the 5 stimulation days compared with sham (two-wavy repeated measure ANOVA, tDCS vs. sham, $p < 0.0001$ and frequency $p < 0.0001$, *post-hoc* Bonferroni's test, tDCS vs. sham, ≧ 1 to < 2 Hz, $p = 0.0004$, ≧ 2 to < 3 Hz, $p = 0.0038$, ≧3 to < 4 Hz, $p = 0.0218$, and ≧ 4 to < 5 Hz, $p = 0.0284$). **C**, Time-frequency spectrogram subtracting the power of sham from tDCS-treated KA rats. Upper panel, sampled and summed every 20 min for post-stim 1-h EEG. Lower panel, selected last 2 min every 30 min for post-stim 1-h EEG. Both including D1 to D5, tDCS, 25 rats; sham, 21 rats. **D**, Post-stimulation delta power (0.1–3.9 Hz) normalized to pre-stimulation compared tDCS and sham (tDCS vs. sham, median power ratio 1.525 vs. 1.153, $U = 5407$, $p = 0.0158$, Mann–Whitney *U* test). **E**, Post-stimulation theta power (4.0–7.9 Hz) normalized to pre-stimulation compared tDCS with sham. **F**, Post-stimulation alpha power (8.0–11.9 Hz) normalized to pre-stimulation compared tDCS with sham. **G**, Post-stimulation beta power (12.0–29.9 Hz) normalized to pre-stimulation compared tDCS with sham (tDCS vs. sham, median power ratio 0.95 vs. 1.080, $U = 5388$, $p = 0.0142$). **H**, Post-stimulation gamma power (30.0–45.0 Hz) normalized to pre-stimulation compared tDCS with sham (tDCS vs. sham, median power ratio 0.892 vs. 1.04, $U = 4665$, $p < 0.0001$). **I**, Correlation between pre-post stimulation change ratios of interictal spike and delta power among all rats (Spearman $r = -0.1670$, $p = 0.0337$), tDCS treated rats ($n = 25$ rats, Spearman $r = -0.1998$, $p = 0.0701$), and sham treated rats ($n = 21$ rats, Spearman $r = -0.06617$, $p = 0.5623$). *p < 0.05, **p < 0.01, ****p < 0.0001.

between tDCS and sham-treated rats (Fig. 5A and B). However, a decrease in hippocampal BDNF protein expression was revealed in tDCS-treated rats compared with sham ($n = 11$ rats in both groups, Mann–Whitney *U* test, $p = 0.028$).

Discussion

Clinical tDCS studies on epilepsy control mostly rely on IED measures. Although interictal spike activity cannot precisely recapitulate seizures, it shares a similar temporal probability distribution and common underlying rhythmicity with seizures and serves as a useful biomarker of cortical excitability [28]. Several studies show that tDCS can reduce IEDs but with large intra- and inter-individual variabilities [16,18]. They also lack long-duration EEG recordings to understand how tDCS modulates brain activity. To this end, our study examined EEG oscillations and interictal spike changes in the pre-post stimulation and subacute follow-up periods to explore whether the epileptic brain responds differently at these stages following stimulation. Our data shows that interictal spikes decreased immediately after c-tDCS but increased in the subsequent two weeks following stimulation withdrawal. The decrease of interictal spike counts could be attributed to the immediate inhibitory effect of c-tDCS, since cathodal DC can hyperpolarize the membrane potential [29,30]. The increase of interictal spikes in the follow-up period may indicate a post-inhibitory rebound (PIR) phenomenon following c-tDCS withdrawal. Hyperpolarization-activated cation currents were reported to cause PIR spikes in the rat medial entorhinal cortex [31]. PIR firing is likely to occur when $GABA_A$ and metabotropic glutamate receptors are simultaneously activated by concurrent excitation and inhibition [32]. Thus, it is reasonable to assume that repeated c-tDCS induced an immediate hyperpolarization and post-stimulation inhibition. The release from prolonged hyperpolarization following withdrawal of inhibitory tDCS consequently activated a PIR-like excitation with increasing interictal spikes in the follow-up period. PIR is reportedly more prevalent during theta (4–6 Hz) than delta oscillation (0.5–2 Hz) in entorhinal cortex interneurons [33]. Whether the increase of interictal spikes in the follow-up period is associated with different oscillation states following stimulation as the oscillation-dependent PIR remains to be determined. c-tDCS generally decreases cortical excitability and induces LTD-like plasticity, while increased cortical excitability and LTP-like plasticity

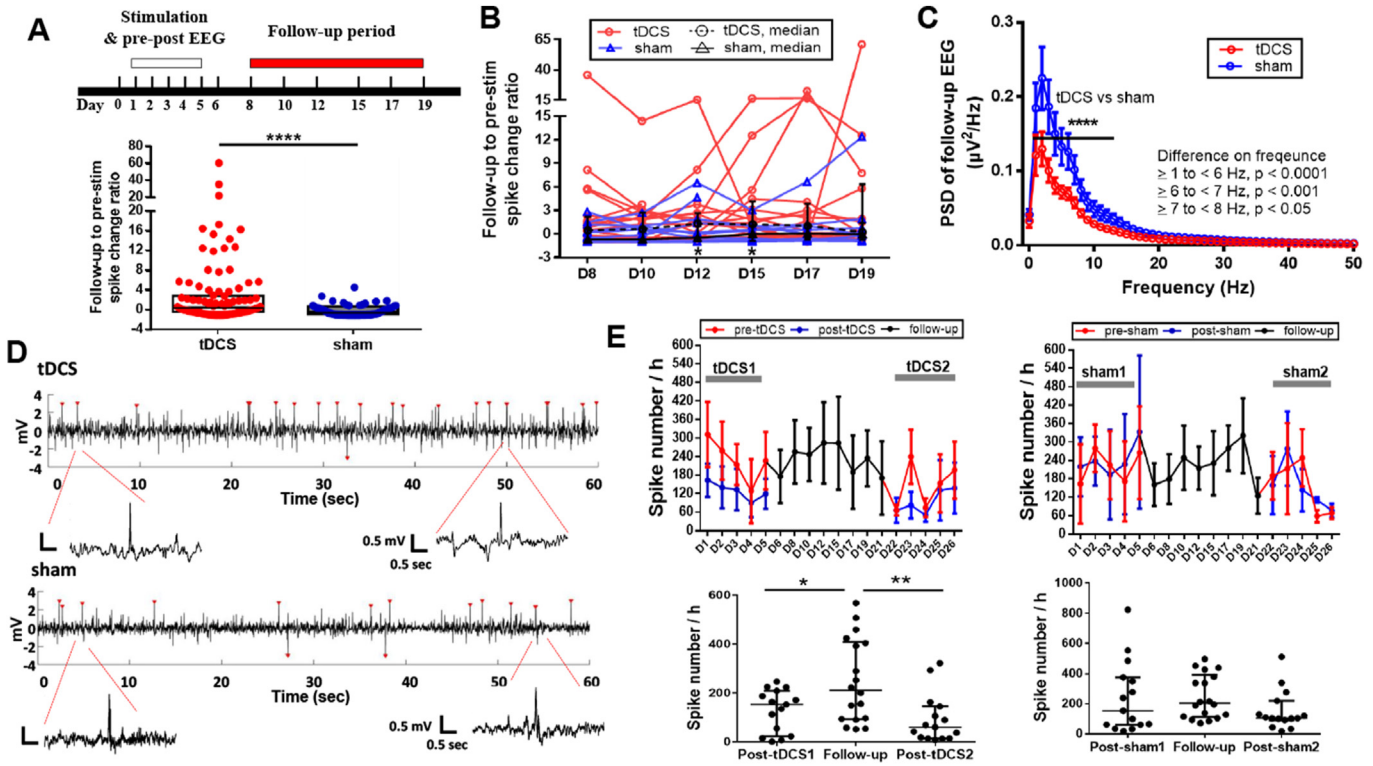

Fig. 3. Increased interictal spikes and decreased low-frequency oscillation in the follow-up period after tDCS withdrawal. A, Experimental time course (upper panel). Red bar indicating the follow-up EEG sampling period. Interictal spike ratio of follow-up to pre-stimulation (D0) in tDCS treated rats compared with sham (lower panel, tDCS vs. sham, median power ratio 0.419 vs. −0.529, $U = 2254$, $p < 0.0001$, Mann−Whitney U test). Dark lines indicate median and boxes for IQR. **B**, Follow-up to pre-stimulation interictal spike ratio in tDCS versus sham on each sampling day. It was significantly increased in tDCS than sham on D12 (tDCS vs. sham, median ratio 1.22 vs. −0.53, $U = 63$, $p = 0.0302$, Mann−Whitney U test) and D15 (tDCS vs. sham, median ratio 0.872 vs. −0.084, $U = 38.00$, $p = 0.0264$). Red line, data of each animal treated with tDCS; blue line, sham. Black traces with triangles and circles represent the median with IQR from sham and tDCS rats, respectively. **C**, PSD of follow-up EEG comparing tDCS and sham (two-way repeated measure ANOVA, tDCS vs. sham, $p < 0.0001$, *post-hoc* Bonferroni's test, tDCS vs. sham, ≧ 1 to < 6 Hz, $p < 0.0001$, ≧ 6 to < 7 Hz, $p = 0.0003$, and ≧ 7 to < 8 Hz, $p = 0.0237$). **D**, Representative interictal spikes in the follow-up period from tDCS treated and sham treated KA rats. **E**, Representative interictal spikes of the follow-up period, and pre- and post-stimulation interictal spikes of the first and second stimulation course of tDCS and sham (upper panel). Statistical graph (lower panel) comparing interictal spike numbers of follow-up period and post-stimulation in tDCS (post-tDCS1 vs. follow-up, $p = 0.0316$, post-tDCS2 vs. follow-up, $p = 0.0036$, one-way ANOVA, $n = 3$ rats) and sham treated rats. pre-stim, pre-stimulation. *$p < 0.05$, **$p < 0.01$,****$p < 0.0001$.

can emerge after certain stimulation protocols, such as 2 mA–20 min over human motor cortex [10]. Another possible explanation for the varying number of interictal spikes at different phases after stimulation is the non-linear long-lasting effect of c-tDCS with LTP-like excitation in the follow-up period after tDCS withdrawal [10,34]. Our results emphasize the importance of monitoring not only immediate inhibitory effects, but also post-stimulation rebound excitation when applying c-tDCS to treat patients with seizure and epilepsy.

EEG oscillations affected by a-tDCS were reported with increased beta and alpha power, varied theta response, and decreased delta power [35–39]. While high frequency gamma oscillations are shown to be enhanced by a-tDCS and decreased by c-tDCS [40], few studies investigated delta oscillations [41]. Our data show that repeated c-tDCS increased low-frequency delta power while decreasing high-frequency gamma and beta power. Interestingly, tDCS modulation on frequency specific EEG oscillations seems to act in a polarity dependent manner. In contrast to a-tDCS which increases high-frequency and reduces low-frequency oscillations [35–39], c-tDCS decreases gamma and beta high-frequency oscillation while enhancing delta low-frequency oscillation. The reduction of gamma high frequency and reinforcement on low frequency oscillation by repeated c-tDCS not only occurred in the KA-induced chronic spontaneous seizure rat model in this study but also in the acute SE rat model [20]. The negative correlation between pre-post stimulation changes of interictal spikes and delta power provides evidence that the decrease of interictal spikes was related to the enhanced delta power immediately after tDCS. Notably, delta oscillation changes exhibit an inverse relationship with the changes in interictal spikes both in acute post-stimulation and subacute follow-up stages, suggesting that delta oscillations underlie the interictal spike changes following tDCS. Delta oscillation in sleep is involved in cognition, seizures and interictal spike activity in epilepsy patients [42], and is inversely correlated with interictal spike activities in drug-resistant epilepsy patients [43]. The role of low-frequency oscillations in modulating epilepsy remains unclear, despite studies showing that low-frequency stimulation can reduce seizure frequency [21,22] and infra-slow (<1 Hz) oscillations can also modulate brain excitability and interictal spike frequency [44,45]. In the sham-controlled pre-post comparison study for tDCS, systemic variables such as anesthesia during stimulation and vigilance state across recordings, which may potentially affect IED and EEG oscillations, were controlled [46–48], showing that the significant delta oscillation increase and the correlated decrease in interictal spikes immediately after stimulation are tDCS-dependent. Our results suggest a possible mechanism for epileptic modulation through c-tDCS by enhancing delta oscillation power, thereby decreasing cortical excitability.

Low seizure severity and low-frequency ictal spike or spike-and-wave complex were observed in tDCS rats while high frequency dense polyspikes were more frequent in sham rats. These ictal EEG features observed in the chronic seizure rat model are similar but

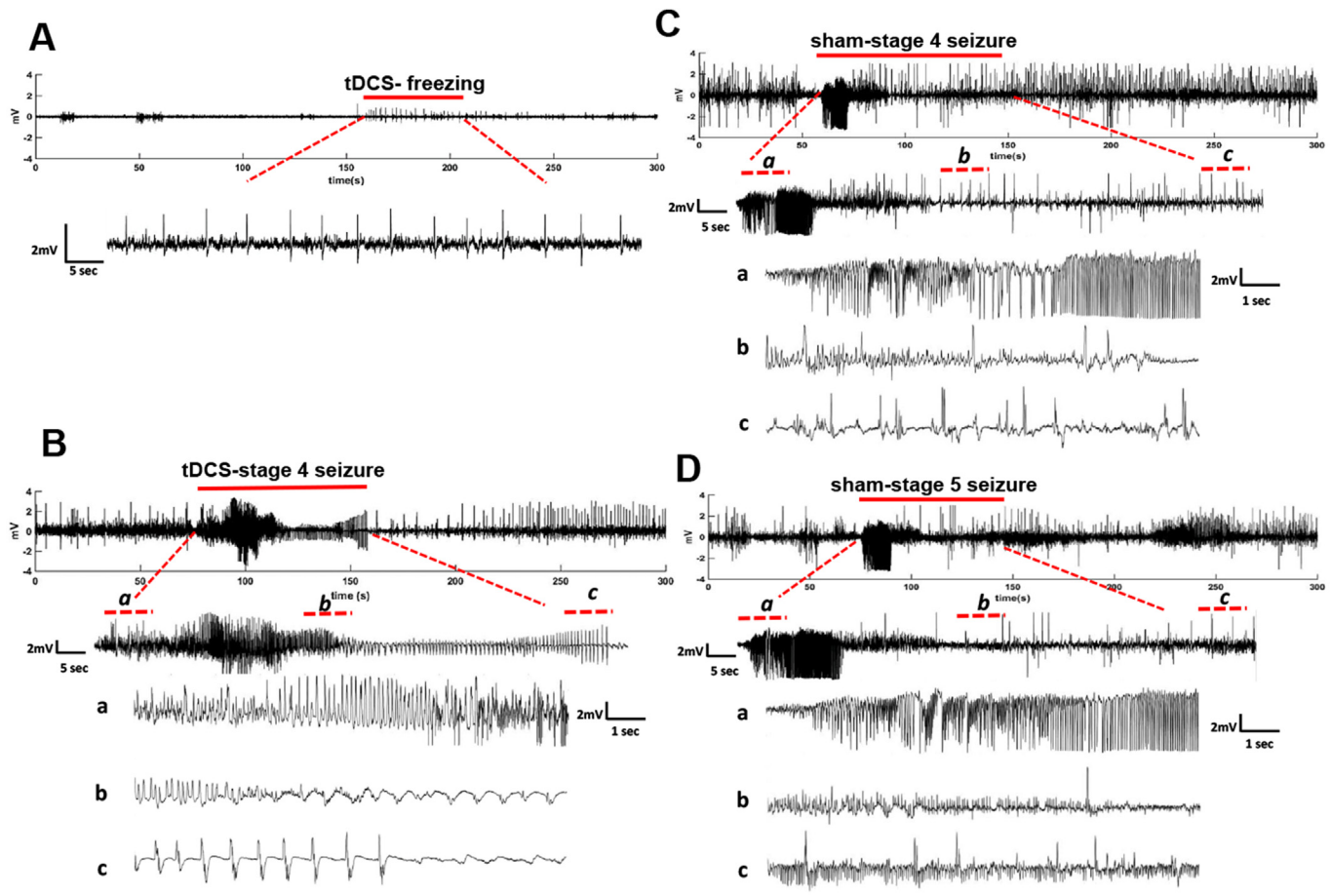

Fig. 4. Ictal discharges in the follow-up period after repeated tDCS. A, Ictal discharge in a post-tDCS treated KA rat presenting freezing seizure. **B**, Ictal discharge in a post-tDCS treated KA rat presenting stage 4 convulsive seizure. **C**, Ictal discharge in a post-sham treated KA rat presenting stage 4 convulsive seizure. **D**, Ictal discharge in a post-sham treated KA rat presenting stage 5 convulsive seizure. Red lines indicate simultaneous ictal EEG segments of the behavior seizures. Red dash lines, zoom-in of *a*, the initial, *b*, the middle and *c*, the end phase of each ictal EEG.

less prominent as those reported in KA-induced SE rats [20]. Unlike the reduction in both hippocampal BDNF protein expression and mossy fiber sprouting in tDCS-treated SE rats, there was a decrease in BDNF protein expression but no changes to mossy fiber sprouting in the rat model of chronic spontaneous seizure following tDCS. The data suggest that the tDCS effect is lower in animals with lower

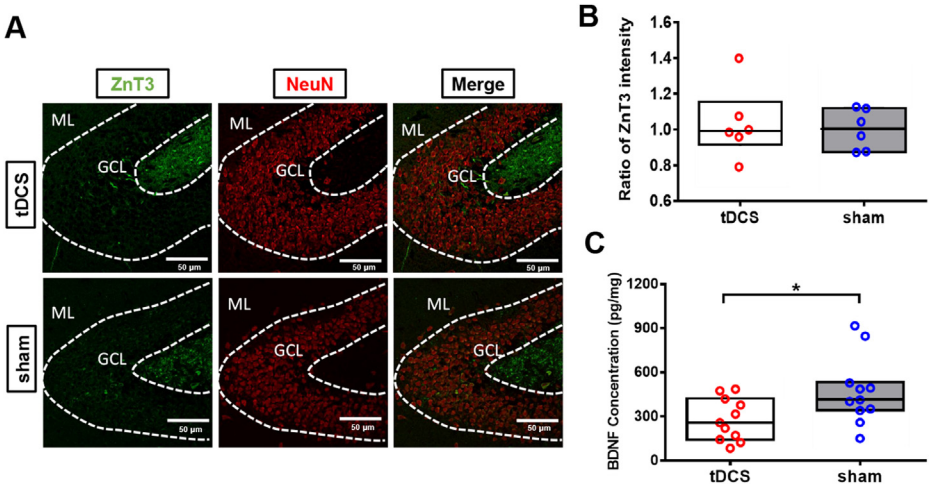

Fig. 5. Hippocampal mossy fiber sprouting and BDNF expression following repeated tDCS. A, DG mossy fiber sproutings by ZnT3 staining in tDCS and sham treated KA rats. **B**, Statistical graph comparing DG mossy fibers of tDCS-treated rats to sham (tDCS $n = 6$, sham $n = 6$, Mann–Whitney U, $p > 0.999$). **C**, Hippocampal BDNF protein expression of tDCS-treated rats compared with sham (Mann–Whitney *U* test, tDCS $n = 11$ vs. sham $n = 11$, median 256.3 vs. 415.4, $U = 27$, $p = 0.028$). Dark lines indicate median and boxes for IQR in B and C. DG, dentate gyrus; GCL, granular cell layer; ML, molecular layer. *$p < 0.05$.

seizure severity, such as the chronic rat model, than in acute SE models.

There are some limitations in the present study. First, the low seizure numbers from the chronic KA rat model make it difficult to obtain the statistical significance of tDCS on behavioral seizure reduction [25]. Second, a tDCS protocol including dosing, duration, session, and the indicated epilepsy type, has not yet been determined. Since we report that tDCS can enhance delta oscillations during stimulation and cause PIR-like firing following stimulation withdrawal, further research should carefully address these issues. Accordingly, clarifying whether severe seizures with high frequency polyspikes would benefit from tDCS more than sporadic seizures with low frequency rhythmic epileptiform discharges could provide important clues for the individualized translation of the non-invasive brain stimulation therapy for epilepsy. Third, the study lacks the mechanism by which tDCS modulates brain oscillations. tDCS traverses through skull to brain, where the current is possibly spreading out or activating connected neural circuits affecting various brain regions, thus dampening the DC focality [49]. Similar to other studies showing that low-frequency stimulation can reduce excitability [50–52], this study further reveals that inducing endogenous low-frequency oscillations, such as delta oscillations, by subthreshold DC stimulation can also decrease neural excitability.

Conclusions

This study shows that c-tDCS can immediately (1) enhance low-frequency oscillations in the delta frequency range, (2) reduce gamma and beta high-frequency oscillations, and (3) generate a significant reduction of interictal spikes in a chronic KA rat model with spontaneous seizures. Following stimulation withdrawal, interictal spike activities rebounded with a concomitant decrease of low-frequency delta and theta oscillations. This inverse relationship between changes of delta oscillations and interictal spikes during on- and off-stimulation suggests that the endogenous delta oscillation enhanced by c-tDCS could underlie the inhibitory effect of tDCS to reduce neural excitability in the epileptic brain.

Author contributions

Y.J.W. and K.S.H. designed research; M.E.C. and Y.J.W. performed research; M.E.C. and Y.J.W. analyzed data; Y.J.W., K.S.H., D.M.D., C.C.C, Y.Z.H., and M.E.C. wrote and revised the paper; D.M.D. and K.S.H. as consultant; Y.J.W. organized the study.

Funding

This work was supported by research grants from the Ministry of Science and Technology, Taiwan (MOST-108-2628-B-006-011, MOST-109-2628-B-006-031), National Cheng Kung University Hospital (NCKUH-10802012, NCKUH-10902013, NCKUH-11001003), in part by Higher Education Sprout Project, Ministry of Education to the Headquarters of University Advancement at National Cheng Kung University, to Yi-Jen Wu and NIH R01 NS114120 01.

Declaration of competing interest

The authors declare no competing interests.

Acknowledgments

We thank the support for imaging analysis from the Core Research Laboratory, and Laboratory Animal Center, College of Medicine, National Cheng Kung University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2021.04.025.

References

[1] Falco-Walter JJ, Scheffer IE, Fisher RS. The new definition and classification of seizures and epilepsy. Epilepsy Res 2018;139:73–9.
[2] Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, et al. ILAE classification of the epilepsies: position paper of the ILAE commission for classification and terminology. Epilepsia 2017;58(4):512–21.
[3] Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. Lancet 2019;393(10172):689–701.
[4] Ryvlin P, Cross JH, Rheims S. Epilepsy surgery in children and adults. Lancet Neurol 2014;13(11):1114–26.
[5] Fisher RS, Velasco AL. Electrical brain stimulation for epilepsy. Nat Rev Neurol 2014;10(5):261–70.
[6] VanHaerents S, Chang BS, Rotenberg A, Pascual-Leone A, Shafi MM. Noninvasive brain stimulation in epilepsy. J Clin Neurophysiol : official publication of the American Electroencephalographic Society 2020;37(2):118–30.
[7] San-Juan D, Morales-Quezada L, Orozco Garduno AJ, Alonso-Vanegas M, Gonzalez-Aragon MF, Espinoza Lopez DA, et al. Transcranial direct current stimulation in epilepsy. Brain stimulation 2015;8(3):455–64.
[8] Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, et al. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol 2005;568(Pt 1):291–303.
[9] Nitsche MA, Paulus W. Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. J Physiol 2000;527(Pt 3):633–9.
[10] Mosayebi Samani M, Agboada D, Jamil A, Kuo MF, Nitsche MA. Titrating the neuroplastic effects of cathodal transcranial direct current stimulation (tDCS) over the primary motor cortex. Cortex; a journal devoted to the study of the nervous system and behavior 2019;119:350–61.
[11] Batsikadze G, Moliadze V, Paulus W, Kuo MF, Nitsche MA. Partially non-linear stimulation intensity-dependent effects of direct current stimulation on motor cortex excitability in humans. J Physiol 2013;591(7):1987–2000.
[12] Cioato SG, Medeiros LF, Marques Filho PR, Vercelino R, de Souza A, Scarabelot VL, et al. Long-lasting effect of transcranial direct current stimulation in the reversal of hyperalgesia and cytokine alterations induced by the neuropathic pain model. Brain stimulation 2016;9(2):209–17.
[13] Wu YJ, Lin CC, Yeh CM, Chien ME, Tsao MC, Tseng P, et al. Repeated transcranial direct current stimulation improves cognitive dysfunction and synaptic plasticity deficit in the prefrontal cortex of streptozotocin-induced diabetic rats. Brain stimulation 2017;10(6):1079–87.
[14] Fregni F, Thome-Souza S, Nitsche MA, Freedman SD, Valente KD, Pascual-Leone A. A controlled clinical trial of cathodal DC polarization in patients with refractory epilepsy. Epilepsia 2006;47(2):335–42.
[15] Auvichayapat N, Rotenberg A, Gersner R, Ngodklang S, Tiamkao S, Tassaneeyakul W, et al. Transcranial direct current stimulation for treatment of refractory childhood focal epilepsy. Brain stimulation 2013;6(4):696–700.
[16] Auvichayapat N, Sinsupan K, Tunkamnerdthai O, Auvichayapat P. Transcranial direct current stimulation for treatment of childhood pharmacoresistant lennox-gastaut syndrome: a pilot study. Front Neurol 2016;7:66.
[17] Tekturk P, Erdogan ET, Kurt A, Vanli-Yavuz EN, Ekizoglu E, Kocagoncu E, et al. The effect of transcranial direct current stimulation on seizure frequency of patients with mesial temporal lobe epilepsy with hippocampal sclerosis. Clin Neurol Neurosurg 2016;149:27–32.
[18] San-Juan D, Espinoza Lopez DA, Vazquez Gregorio R, Trenado C, Fernandez-Gonzalez Aragon M, Morales-Quezada L, et al. Transcranial direct current stimulation in mesial temporal lobe epilepsy and hippocampal sclerosis. Brain stimulation 2017;10(1):28–35.
[19] Yang D, Wang Q, Xu C, Fang F, Fan J, Li L, et al. Transcranial direct current stimulation reduces seizure frequency in patients with refractory focal epilepsy: a randomized, double-blind, sham-controlled, and three-arm parallel multicenter study. Brain stimulation 2020;13(1):109–16.
[20] Wu YJ, Chien ME, Huang CH, Chiang CC, Lin CC, Huang CW, et al. Transcranial direct current stimulation alleviates seizure severity in kainic acid-induced status epilepticus rats. Exp Neurol 2020;328:113264.
[21] Toprani S, Durand DM. Long-lasting hyperpolarization underlies seizure reduction by low frequency deep brain electrical stimulation. J Physiol 2013;591(22):5765–90.

[22] Ladas TP, Chiang CC, Gonzalez-Reyes LE, Nowak T, Durand DM. Seizure reduction through interneuron-mediated entrainment using low frequency optical stimulation. Exp Neurol 2015;269:120–32.
[23] Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr Clin Neurophysiol 1972;32(3):281–94.
[24] Sharma S, Puttachary S, Thippeswamy A, Kanthasamy AG, Thippeswamy T. Status epilepticus: behavioral and electroencephalography seizure correlates in kainate experimental models. Front Neurol 2018;9:7.
[25] Drexel M, Preidt AP, Sperk G. Sequel of spontaneous seizures after kainic acid-induced status epilepticus and associated neuropathological changes in the subiculum and entorhinal cortex. Neuropharmacology 2012;63(5):806–17.
[26] Barbati SA, Cocco S, Longo V, Spinelli M, Gironi K, Mattera A, et al. Enhancing plasticity mechanisms in the mouse motor cortex by anodal transcranial direct-current stimulation: the contribution of nitric oxide signaling. Cerebr Cortex 2020;30(5):2972–85.
[27] Liebetanz D, Klinker F, Hering D, Koch R, Nitsche MA, Potschka H, et al. Anticonvulsant effects of transcranial direct-current stimulation (tDCS) in the rat cortical ramp model of focal epilepsy. Epilepsia 2006;47(7):1216–24.
[28] Karoly PJ, Freestone DR, Boston R, Grayden DB, Himes D, Leyde K, et al. Interictal spikes and epileptic seizures: their relationship and underlying rhythmicity. Brain : J Neurol 2016;139(Pt 4):1066–78.
[29] Rahman A, Reato D, Arlotti M, Gasca F, Datta A, Parra LC, et al. Cellular effects of acute direct current stimulation: somatic and synaptic terminal effects. J Physiol 2013;591(10):2563–78.
[30] Voroslakos M, Takeuchi Y, Brinyiczki K, Zombori T, Oliva A, Fernandez-Ruiz A, et al. Direct effects of transcranial electric stimulation on brain circuits in rats and humans. Nat Commun 2018;9(1):483.
[31] Ferrante M, Shay CF, Tsuno Y, William Chapman G, Hasselmo ME. Post-inhibitory rebound spikes in rat medial entorhinal layer II/III principal cells: in vivo, in vitro, and computational modeling characterization. Cerebr Cortex 2017;27(3):2111–25.
[32] Zheng N, Raman IM. Prolonged postinhibitory rebound firing in the cerebellar nuclei mediated by group I metabotropic glutamate receptor potentiation of L-type calcium currents. J Neurosci : the official journal of the Society for Neuroscience 2011;31(28):10283–92.
[33] Adhikari MH, Quilichini PP, Roy D, Jirsa V, Bernard C. Brain state dependent postinhibitory rebound in entorhinal cortex interneurons. J Neurosci : the official journal of the Society for Neuroscience 2012;32(19):6501–10.
[34] Shilo G, Lavidor M. Non-linear effects of cathodal transcranial direct current stimulation (tDCS) of the primary motor cortex on implicit motor learning. Exp Brain Res 2019;237(4):919–25.
[35] Straudi S, Bonsangue V, Mele S, Craighero L, Montis A, Fregni F, et al. Bilateral M1 anodal transcranial direct current stimulation in post traumatic chronic minimally conscious state: a pilot EEG-tDCS study. Brain Inj 2019;33(4):490–5.
[36] Saadi ZK, Saadat M, Kamali AM, Yahyavi SS, Nami M. Electrophysiological modulation and cognitive-verbal enhancement by multi-session Broca's stimulation: a quantitative EEG transcranial direct current stimulation based investigation. J Integr Neurosci 2019;18(2):107–15.
[37] Song M, Shin Y, Yun K. Beta-frequency EEG activity increased during transcranial direct current stimulation. Neuroreport 2014;25(18):1433–6.
[38] Accornero N, Capozza M, Pieroni L, Pro S, Davi L, Mecarelli O. EEG mean frequency changes in healthy subjects during prefrontal transcranial direct current stimulation. J Neurophysiol 2014;112(6):1367–75.
[39] Roy A, Baxter B, He B. High-definition transcranial direct current stimulation induces both acute and persistent changes in broadband cortical synchronization: a simultaneous tDCS-EEG study. IEEE Trans Biomed Eng 2014;61(7):1967–78.
[40] Reato D, Rahman A, Bikson M, Parra LC. Low-intensity electrical stimulation affects network dynamics by modulating population rate and spike timing. J Neurosci : the official journal of the Society for Neuroscience 2010;30(45):15067–79.
[41] Das S, Holland P, Frens MA, Donchin O. Impact of transcranial direct current stimulation (tDCS) on neuronal functions. Front Neurosci 2016;10:550.
[42] Boly M, Jones B, Findlay G, Plumley E, Mensen A, Hermann B, et al. Altered sleep homeostasis correlates with cognitive impairment in patients with focal epilepsy. Brain : J Neurol 2017;140(4):1026–40.
[43] Zubler F, Rubino A, Lo Russo G, Schindler K, Nobili L. Correlating interictal spikes with sigma and delta dynamics during non-rapid-eye-movement-sleep. Front Neurol 2017;8:288.
[44] Vanhatalo S, Palva JM, Holmes MD, Miller JW, Voipio J, Kaila K. Infraslow oscillations modulate excitability and interictal epileptic activity in the human cortex during sleep. Proc Natl Acad Sci U S A 2004;101(14):5053–7.
[45] Lundstrom BN, Boly M, Duckrow R, Zaveri HP, Blumenfeld H. Slowing less than 1 Hz is decreased near the seizure onset zone. Sci Rep 2019;9(1):6218.
[46] von Ellenrieder N, Koupparis A, Gotman J. Interactions of interictal epileptic discharges with sleep slow waves and spindles. Brain : J Neurol 2020;143(4):e27.
[47] Sedigh-Sarvestani M, Thuku GI, Sunderam S, Parkar A, Weinstein SL, Schiff SJ, et al. Rapid eye movement sleep and hippocampal theta oscillations precede seizure onset in the tetanus toxin model of temporal lobe epilepsy. J Neurosci : the official journal of the Society for Neuroscience 2014;34(4):1105–14.
[48] Colom LV, Garcia-Hernandez A, Castaneda MT, Perez-Cordova MG, Garrido-Sanabria ER. Septo-hippocampal networks in chronically epileptic rats: potential antiepileptic effects of theta rhythm generation. J Neurophysiol 2006;95(6):3645–53.
[49] Jackson MP, Rahman A, Lafon B, Kronberg G, Ling D, Parra LC, et al. Animal models of transcranial direct current stimulation: methods and mechanisms. Clin Neurophysiol : official journal of the International Federation of Clinical Neurophysiology 2016;127(11):3425–54.
[50] Rashid S, Pho G, Czigler M, Werz MA, Durand DM. Low frequency stimulation of ventral hippocampal commissures reduces seizures in a rat model of chronic temporal lobe epilepsy. Epilepsia 2012;53(1):147–56.
[51] Koubeissi MZ, Kahriman E, Syed TU, Miller J, Durand DM. Low-frequency electrical stimulation of a fiber tract in temporal lobe epilepsy. Ann Neurol 2013;74(2):223–31.
[52] Couturier NH, Durand DM. Corpus callosum low-frequency stimulation suppresses seizures in an acute rat model of focal cortical seizures. Epilepsia 2018;59(12):2219–30.