Brain Stimulation 12 (2019) 1429–1438

Contents lists available at ScienceDirect

Brain Stimulation

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

Temporally irregular electrical stimulation to the epileptogenic focus delays epileptogenesis in rats

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ARTICLE INFO

Article history:
Received 31 January 2019
Received in revised form
8 July 2019
Accepted 23 July 2019
Available online 25 July 2019

Keywords:
Epilepsy
Deep brain stimulation
Kindling
Epileptogenesis
Temporally irregular stimulation
Random inter-pulse-interval

ABSTRACT

Background: Variation in the temporal patterns of electrical pulses in stimulation trains has opened a new field of opportunity for the treatment of neurological disorders, such as pharmacoresistant temporal lobe epilepsy. Whether this novel type of stimulation affects epileptogenesis remains to be investigated.
Objective: The purpose of this study was to analyze the effects of temporally irregular deep brain stimulation on kindling-induced epileptogenesis in rats.
Methods: Temporally irregular deep brain stimulation was delivered at different times with respect to the kindling stimulation. Behavioral and electrographic changes on kindling acquisition were compared with a control group and a temporally regular deep brain stimulation-treated group. The propagation of epileptiform activity was analyzed with wavelet cross-correlation analysis, and interictal epileptiform discharge ratios were obtained.
Results: Temporally irregular deep brain stimulation delivered in the epileptogenic focus during the interictal period shortened the daily afterdischarge duration, slowed the progression of seizure stages, diminished the generalized seizure duration and interfered with the propagation of epileptiform activity from the seizure onset zone to the ipsi- and contralateral motor cortex. We also found a negative correlation between seizure severity and interictal epileptiform discharges in rats stimulated with temporally irregular deep brain stimulation.
Conclusion: These results provide evidence that temporally irregular deep brain stimulation interferes with the establishment of epilepsy by delaying epileptogenesis by almost twice as long in kindling animals. Thus, temporally irregular deep brain stimulation could be a preventive approach against epilepsy.

Introduction

Epilepsy is a common neurological disorder with a prevalence of 6.4 cases per 1000 persons. Approximately one-third of patients with epilepsy do not respond to conventional treatment with antiepileptic drugs, and surgical resection of the epileptic foci is a viable alternative for only a minority of patients [1]. Temporal lobe epilepsy is the most frequent type of partial epilepsy and has high rates of intractability [2]; thus, it is important to develop different effective strategies for its treatment.

Deep brain electrical stimulation (DBS) is an alternative therapy used to treat some pharmacoresistant neurological disorders; however, optimal stimulation parameters for treating epilepsy have not yet been established [3]. DBS can be classified into open-loop and closed-loop (also known as adaptive) paradigms. Closed-loop DBS employs a sensor to record a signal (biomarker) linked to symptoms, while open-loop DBS stimulation parameters, including duration, amplitude, and frequency of the pulse train, remain constant regardless of fluctuations in the disease state. Engineering innovations and techniques are currently focused on the development of innovative electrode designs and computational models to guide stimulation and modify temporal patterns of stimulation to improve therapeutic outcomes [4,5]. Temporal pattern variations of the electrical pulses in stimulation trains is an emerging proposal

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https://doi.org/10.1016/j.brs.2019.07.016
1935-861X/

that has opened a new opportunity to improve stimulation effectiveness, minimize its side effects and reduce the energy consumption of DBS devices [4,6].

Temporally irregular DBS (TiDBS) patterns have been tested in humans with Parkinson's disease and have resulted in positive outcomes when the instantaneous pulse-frequency distribution of the stimulation train was in the range of clinically effective frequencies [7,8]. Nonperiodic stimulation [9,10] and Poisson-distributed stimulation [11,12] have been tested in rodents treated with pentylenetetrazol, kainic acid and pilocarpine with positive outcomes; however, their effects on epileptogenesis remain unknown. This is a critical period in which TiDBS may exert a more robust effect.

Traditionally, epileptic tissue has been considered to be in a pathological hyperexcitable and hypersynchronous state during seizures [13]. In accordance with this hypothesis, it has been proposed that irregular patterns of stimulation may interrupt seizures by interfering with seizure synchronization [9]; however, recent research has shown that neural firing does not synchronize until the end of the seizure within the seizure onset zone [14]. Thus, synchronization may facilitate seizure termination instead of provocation [15]. We hypothesize that, somehow, TiDBS may favor synchronicity when applied at specific moments in the seizure onset zone and consequently facilitate seizure termination. If this is true, is it possible that TiDBS also has protective effects against epileptogenesis? This is a question that, to our knowledge, remains unanswered.

The aim of this work was to investigate the effects of TiDBS on epileptogenesis in rats. To achieve this goal, we designed TiDBS with instantaneous pulse frequencies ranging from 1 to 100 Hz, which is the frequency range that has shown some protective effects against seizures in animal models [16]. We used electrical amygdala kindling (AK), a well-established animal model of temporal lobe epileptogenesis [17], characterized by the progressive development of electrographic afterdischarge (AD) in response to daily repetitive electrical stimulation of the amygdala. The repetitive stimulation produces an enhancement of the cortical activity accompanied by the development of generalized tonic-clonic seizures [18]. AK provides temporal and spatial control over the generation of an epileptogenic focus.

Materials and methods

Animals

Wistar rats (male, 290–310 g) were used in this study. All animals were maintained in individual cages with a 12 h/12 h light/dark cycle (lights on from 8:00 to 20:00) and with food and water ad libitum. All experiments were conducted in accordance with the Mexican Official Norm (NOM-062 ZOO-1999) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the ethics committee of the Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz.

Surgery

Animals were anesthetized with a ketamine/xylazine mixture (100/10 mg/kg, i.m.), mounted on a stereotaxic frame (model 1430; David Kopf, Tujunga, CA) and implanted with two epidural electrodes in each motor cortex (AP 2.0; L ± 3.0) for electrocorticographic recording and a tripolar electrode in the right basolateral amygdala (BLA) (AP -2.4 mm; L 4.8 mm; V −8.7 mm) for stimulation and depth electroencephalographic recording. Two screws were placed in the bone over the cerebellum to serve as grounds and references, and two additional screws served as anchors (Fig. 1). Epidural electrodes implanted in the motor cortices were made of 500 μm stainless-steel wires, and the electrodes implanted in the BLA were made of three 254 μm diameter stainless-steel wires glued with 500 μm between each tip; two tips were used for stimulation and another was used for recording. Electrode leads were connected to a miniature receptacle, which was attached to the skull with dental cement. The coordinates were measured from bregma according to the atlas of Paxinos & Watson [19]. After the behavioral studies, the electrode locations were histologically verified.

Amygdaloid kindling

After 7–8 days of recovery from surgery, the AD threshold of each rat was determined by delivering electrical stimuli to the BLA (monophasic square-wave pulses, 60 Hz, 1 ms pulse, 1 s train) using an S88x constant current stimulator (Grass, Massachusetts). The stimulation intensity started at 50 μA and then increased in 50 μA steps every 5 min. The minimal intensity that produced at least a 5 s AD was designated as the AD threshold for that rat and was subsequently used for kindling stimulation. The AK stimulus was delivered once every 24 h until the animals exhibited three consecutive stage 5 seizures according to the Racine scale [20]; they were then regarded as fully kindled, and the AK experiments were concluded.

Deep brain stimulation

For the construction of the TiDBS pattern (Fig. 2A), a pseudorandom sequence generator was designed and built using a linear shift feedback register on a microcontroller (Fig. 2B). The generated binary sequences exhibit random properties but have a finite length (2^{24}–1); therefore, they are deterministic. The sequences were then converted to timer data to generate the pseudorandom intervals between pulses. The device was programmed to deliver stimulation trains with a range from 1 to 100 Hz, a mean instantaneous pulse frequency of 31 Hz (Fig. 2C), a mean pulse rate of 4 Hz (Fig. 2D) and an entropy [21] of 10.4 bits/pulse of a simulation of a stimulation train. The instantaneous pulse frequency is defined as the reciprocal of the period between two adjacent pulses, and the pulse rate is defined as the number of pulses delivered every second. The TiDBS distribution is fitted by an exponential decay equation, $y = 34.17\, e^{-0.009x} + 1.54$ (red line in Fig. 2B), as revealed by nonlinear regression analysis (Fig. 2B). The mean pulse rate of the TiDBS was selected to be the same as that of the temporally regular DBS (TrDBS) (Fig. 2B and C)..

TiDBS and TrDBS (biphasic square-wave pulses, 100 μs pulse and 20 min train) were delivered to the BLA through the same electrodes used for AK. The train duration was chosen based on previous works that describe the beneficial effects of DBS on AK with trains of 15–30 min [22]. The intensity for both TiDBS and TrDBS was determined for each rat after the determination of the AD threshold. The rats were stimulated with a train of 500 s at an initial intensity of 100 μA; the intensity was subsequently increased by 50 μA steps every 5 min until subconvulsive behaviors, such as eyeblink and facial twitching, were observed. Then, this value was reduced by 100 μA to avoid abnormal behaviors and guarantee amygdala stimulation [11,12,23]. As with AK, DBS was delivered once every 24 h from the beginning to the end of the experiments.

Experimental design

Two experiments were performed. First, the effect of TiDBS delivered at different moments with respect to kindling stimulation was investigated. Rats were randomly divided into three groups.

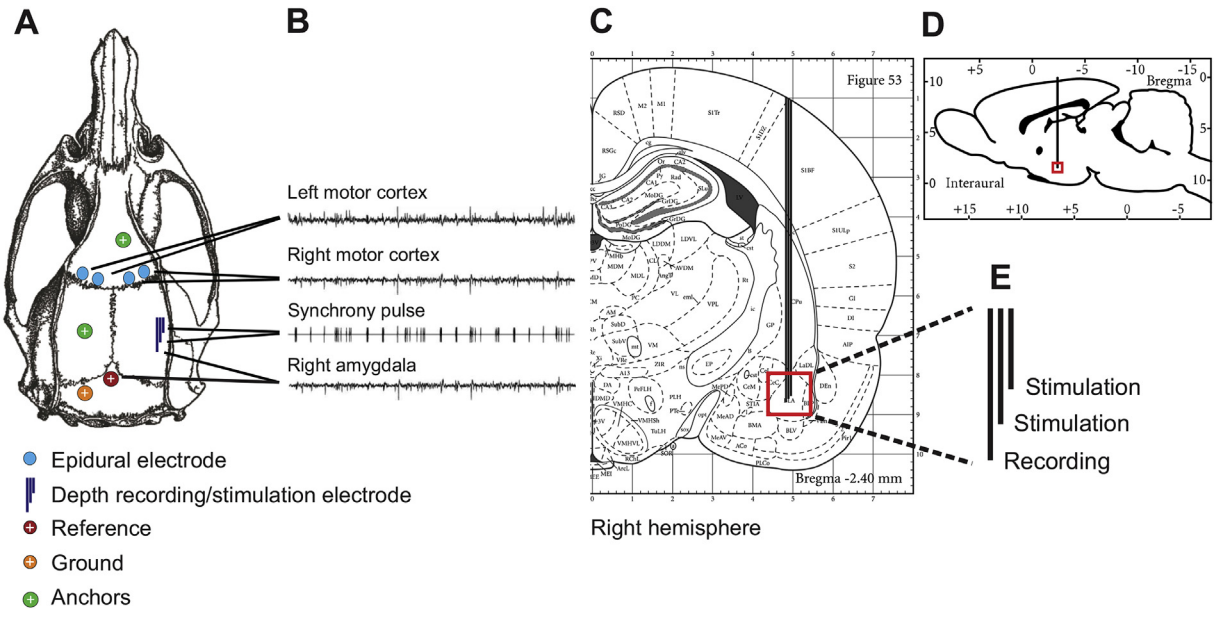

Fig. 1. Experimental preparation. (A) Schematic overview of the configuration of electrode implantation: a tripolar recording/stimulation depth electrode in the right basolateral amygdala (AP -2.4 mm; L 4.8 mm; V −8.7 mm relative to bregma [19]), 2 epidural electrodes over the dura of each motor cortex, 2 screws over the cerebellum for reference and ground and two additional screws placed in the frontal and parietal bone to serve as anchors. (B) Representative electrocorticograms from motor cortices depth electroencephalographic recording from the amygdala and the synchrony pulse during TiDBS. (C and D) Schematic representations of coronal and sagittal views (respectively) from an atlas showing the region in which the tips of the electrodes were placed. (E) Electrode configuration of the tripolar electrode with two stimulation tips and one recording tip. Abbreviations: TiDBS, temporally irregular DBS.

Animals received TiDBS prior to the AK stimulus (TiDBS + K, n = 9), immediately after the AK stimulus (K + TiDBS-R, n = 9) and 5 min after the termination of the AD (K + TiDBS, n = 9). In the second experiment, we compared the effects of TiDBS and TrDBS. To that end, following data analysis on the results of the first experiment, the K + TiDBS condition showed protective effects. It was then compared with another experimental group in which temporally regular DBS was applied 5 min after the termination of the AD (TrDBS, n = 9). Additional rats were assigned to a control group (n = 9) that only received AK stimuli (Fig. 3).

Analyses

Analysis of kindling-induced epileptogenesis

All animal behavior was recorded on videotape during AK and behavioral seizures. The videos were analyzed offline by two blinded observers, and the kindling progression was scored according to the Racine scale [20]: stage 1, facial movement; stage 2, head nodding; stage 3, unilateral forelimb clonus; stage 4, bilateral forelimb clonus and rearing; and stage 5, bilateral forelimb clonus, rearing and falling. Seizure stages 1–3 represent complex focal seizures, while stages 4–5 are generalized seizures [24]. Seizure stages and afterdischarge duration (ADD) evoked by kindling stimulation, which represent the severity of seizures, were measured daily. To further analyze the stepwise progression of the kindling, we calculated the cumulative number of stimulations required for the rats to reach and remain at each seizure stage. Additionally, the generalized seizure duration (GSD), defined as the duration of bilateral forelimb clonus, was analyzed as previously described [25]. When the animals exhibited three consecutive stage 5 seizures, they were considered fully kindled.

Electrophysiological recordings

The recordings from the BLA and motor cortices were amplified and bandpass filtered between 1 Hz and 100 Hz by a 7P511 amplifier (Grass, Massachusetts). Signals were digitized at a rate of 1000 Hz and stored on a hard drive.

Wavelet cross-correlation analysis

To analyze the effects of TiDBS on epileptiform propagation from the BLA to the ipsi- and contralateral motor cortex, we used wavelet cross-correlation analysis, a method that has been used to assess the relationship between nonstationary signals such as electroencephalographic recordings of seizures (Y. Mizuno-Matsumoto et al., 2005; Yuko Mizuno-Matsumoto et al., 2005; Yuko Mizuno-Matsumoto, Motamedi, Webber, & Lesser, 2002). This spectral analysis is suitable for nonstationary signals with abrupt fluctuations, such as those observed in an epileptic seizure (Mizuno-matsumoto, Motamedi, Kaishima, Shinosaki, & Lesser, 2005). The wavelet cross-correlation coefficients (WCC) from the right BLA to right MC, the right BLA to left MC and the right BLA to left MC of each AD recording were computed in the frequency band of 1–50 Hz. The WCC values from the K + TiDBS, TiDBS + K, and control groups were compared.

To ensure that the recordings from all rats had the same mean voltage, signals were normalized using the following equation:

$$nS(t) = s(t) * \frac{\sqrt{\frac{\sum_{i=1}^{N} rs_i^2}{N}}}{\sqrt{\frac{\sum_{i=1}^{N} s_i^2}{N}}}$$

where nS is the normalized signal, t is time, s is the non-normalized signal, rs is the reference signal and N is the total number of samples in each signal. A 10 s baseline recording from a random rat without any artifacts was selected to be rs. Using this equation, we ensure that the baseline recordings of all rats had the same RMS.

Wavelet transforms were computed for each recording using Morlet as the mother wavelet with twelve voices per octave. The WCCs were obtained using the following equation [26]:

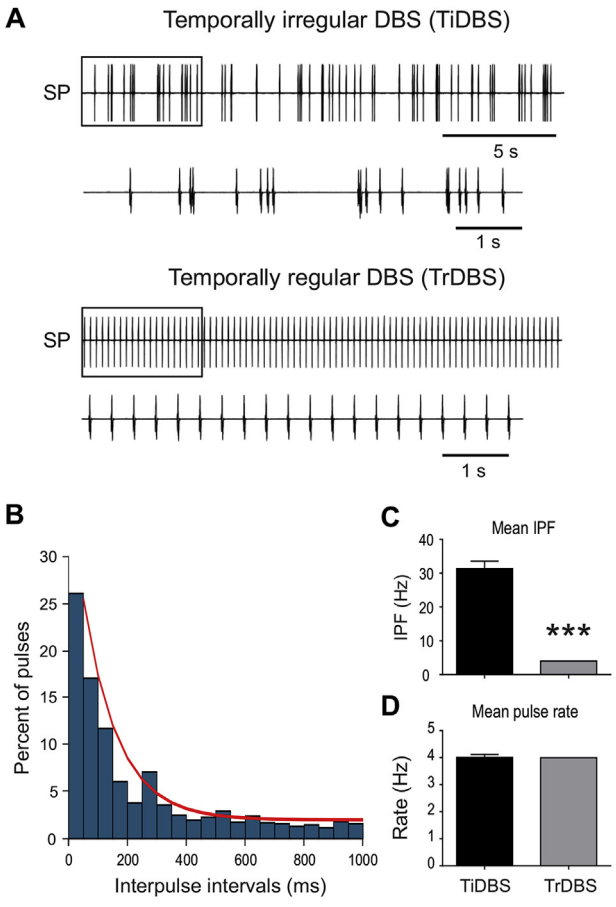

Fig. 2. (A) Twenty seconds of representative recordings of temporally irregular DBS (TiDBS) and temporally regular DBS (TrDBS). The rectangles highlight 5-s zoomed-in epochs. (B) Curve fitting using nonlinear regression analysis for the distribution of the interpulse intervals of the TiDBS. The histogram is fitted by the exponential decay equation $y = 34.17\ e^{-0.009x} + 1.54$. Pearson's correlation coefficient test, n = 20, r = 0.989 (red line). Comparison of the mean IPF (C) and the mean pulse rate (D) of the TiDBS and TrDBS. Unpaired Student's *t*-test was used for C and D. n = 1382 for each group. ***p < 0.0001 for C, and p = 0.965 for D. Abbreviations: IPF, instantaneous pulse frequency; SP, synchrony pulse. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$$WCC(a,\tau) = \left| \sum_{b=1}^{N} W_1^*(a,b) * \sum_{b=1}^{N} W_2(a,b-\tau) \right|$$

where a is the wavelet scale, τ is the time delay, $W_1{}^*$ is the complex conjugate of the continuous wavelet transform of the first signal, W_2 is the continuous wavelet transform of the second signal, and b is the translation unit of the wavelet. For the analysis over the 1–50 Hz band, the following equation was used:

$$BWC = \sum_{a=l}^{u} \frac{\sum_{b=1}^{N} WCC(a,b)}{N}$$

where *BWC* is the band-specific WCC, and l and u are the lower and upper boundaries of the bandwidth of choice, respectively.

These analyses were implemented with custom routines written in MATLAB (The Mathworks Inc., Natick, MA).

Interictal epileptiform discharge ratio

All previous analyses have focused on the ictal period; however, after data analysis, we observed that TiDBS delayed epileptogenesis only when applied during the interictal period. Therefore, to assess the effects of TiDBS on the interictal period, the interictal epileptiform discharges (IEDs) were evaluated. We counted the number of IEDs during the 5 min following the termination of the AD of stage 5 seizures. The IEDs were counted with the MATLAB function *findpeaks.m*. Every detected event was analyzed visually. We considered a complete IED from the first deflection from baseline to the return to baseline. We then computed the IED ratio (the total number of IEDs divided over time), followed by a correlation analysis between the transformed IED ratio and the mean GSD in the control, K + TiDBS and TiDBS + K groups. The ratio was transformed by raising it to the fourth power to perform correlation analysis (See *Statistics* below).

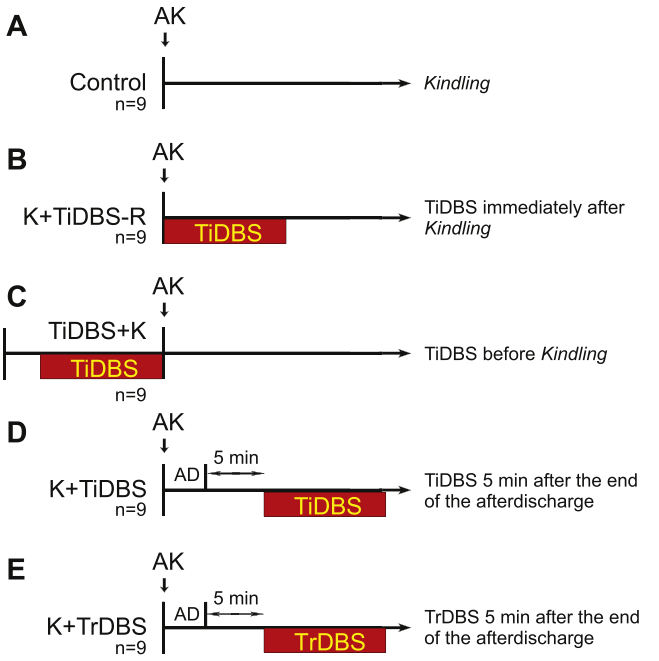

Fig. 3. Experimental groups and timelines of each stimulation condition for Experiment 1(A–D) and Experiment 2 (E). Temporally irregular or regular deep brain stimulation (biphasic square-wave pulses, 0.1 ms/pulse and 20 min train) was delivered at different times with respect to the kindling stimulation. Kindling stimulation and DBS were applied once every 24 h until the animals exhibited the fully kindled stage. Abbreviations: AD, afterdischarge; AK, electrical amygdala kindling; TiDBS, temporally irregular deep brain stimulation; TrDBS, temporally regular deep brain stimulation.

Statistics

The Shapiro-Wilk test was used to evaluate whether the assumptions of normality were met for all measures. Levene's test was used to assess the equality of variances when two or more groups were compared. Student's *t*-test was used to assess the differences between the parameters of the TiDBS and TrDBS trains. An analysis of group differences in kindling acquisition was performed by two-way analysis of variance (ANOVA) for repeated measures and Bonferroni's post hoc test. Comparisons of the number of stimulations for each seizure stage during kindling acquisition and the GSD were made with one-way ANOVA and Bonferroni's post hoc test. The WCC was analyzed by one-way ANOVA for repeated measures and Bonferroni's post hoc test. Pearson's linear correlation was used to analyze the associations between the IED ratios. Data are presented as the mean ± S.E.M. Statistical analysis was carried out using the statistical packages of SPSS (V20.0, IBM Corporation, New York, USA). For all analyses, $p < 0.05$ was considered significant.

Table 1
AD threshold amplitude for AK stimulus and intensity delivered for DBS.

Group	AD threshold (μA)	DBS (μA)
	Mean ± SD	Mean ± SD
Control	180.56 ± 39.09	
K + TiDBS	213.89 ± 65.09	127.78 ± 36.32
TiDBS + K	233.33 ± 57.28	133.33 ± 35.36
K + TiDBS-R	186.67 ± 48.48	122.22 ± 36.32
K + TrDBS	207.22 ± 58.80	127.78 ± 36.32
All groups	204.3 ± 10.1	127.78 ± 36.1

No significant differences were found among the groups (two-way ANOVA followed by Bonferroni's post-hoc tests).

Results

Afterdischarge threshold and DBS intensity

The mean AD threshold amplitude for the AK stimulus was 204.3 ± 10.1 μA, and the mean intensity for DBS was 127.78 ± 36.1 μA. The intensities delivered for AK and DBS per group are shown in Table 1. No significant differences were found among the groups.

Experiment 1. effects of TiDBS on kindling epileptogenesis

Application of K + TiDBS and TiDBS + K at the kindling focus reduced the mean ADD ($p < 0.001$ and $p < 0.05$, respectively; Fig. 4A) and delayed the behavioral progression of seizure stages ($p < 0.001$ and $p < 0.005$, respectively; Fig. 4B) compared to the control group. K + TiDBS-R had no effect on seizure stage (Fig. 4B). Unfortunately, ADDs were not analyzed for this group because stimulation artifacts masked the AD recordings. The K + TiDBS and TiDBS + K groups also showed a decrease in the seizure stage compared to the K + TiDBS-R group ($p < 0.001$ for both, Fig. 4A and B). No significant differences were noted when comparing K + TiDBS and TiDBS + K (Fig. 4A and B).

We further calculated the number of stimulations needed to reach and remain at each seizure stage. Compared to the control and K + TiDBS-R groups, rats in the K + TiDBS group remained in stage 1 ($p < 0.001$ and $p < 0.01$, respectively; Fig. 4C) and stage 2 ($p < 0.05$ and $p < 0.01$, respectively; Fig. 4C) for more days; they also needed more days of stimulation to reach each stage and become fully kindled ($p < 0.01$ for both; Fig. 4D). TiDBS + K increased the number of days that animals remained in stage 1 compared with the control and K + TiDBS-R groups ($p < 0.01$ for both; Fig. 4C). It also increased the number of days to reach each stage and the fully kindled state compared to the control and K + TiDBS-R groups ($p < 0.05$ and $p < 0.01$, respectively; Fig. 4D). K + TiDBS-R had no effect on the number of stimulations needed to reach and remain at

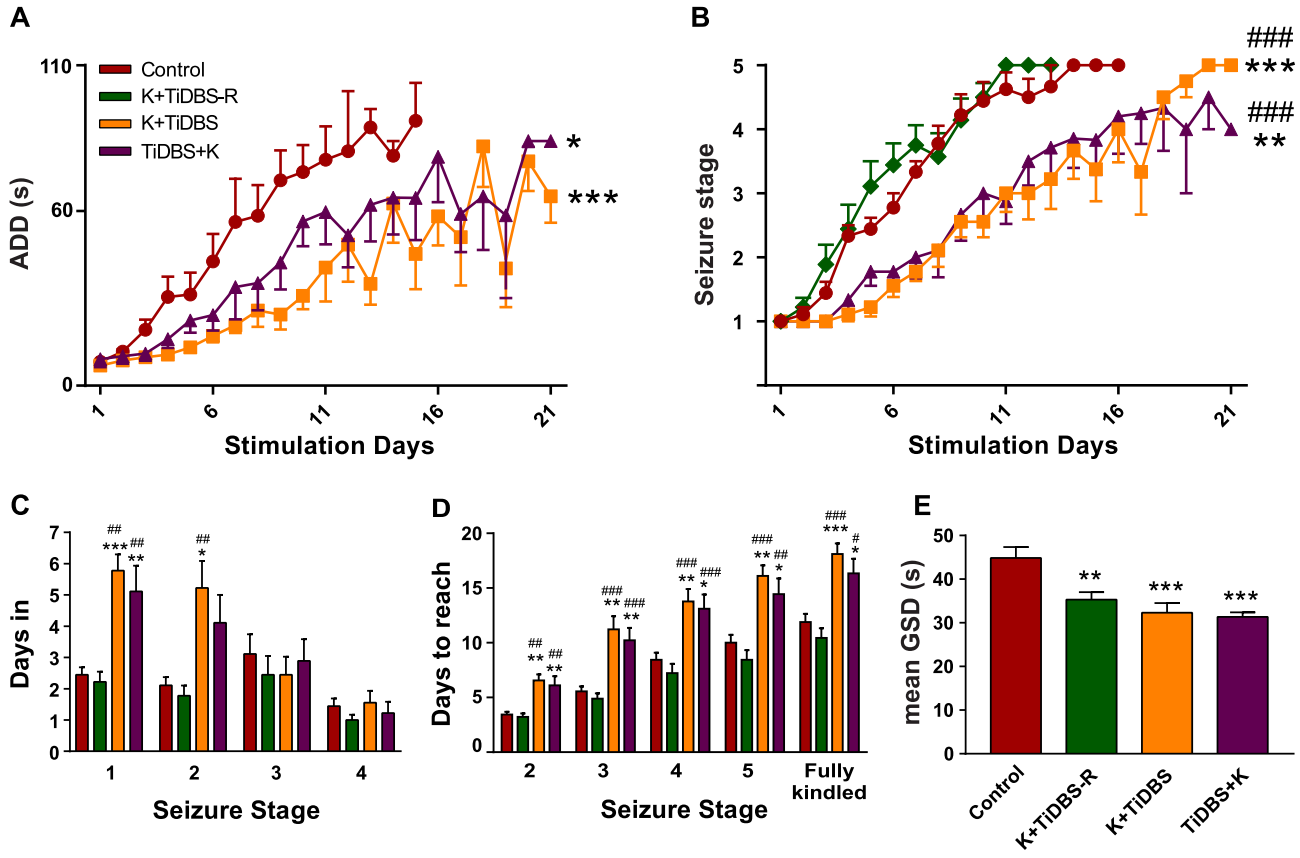

Fig. 4. TiDBS delivered to the kindling focus delays kindling-induced epileptogenesis in rats. Effects of different conditions of TiDBS on daily ADD (A), seizure stages (B), days of stimulation at each stage, (D) days of stimulation required to reach each stage during kindling acquisition, and (E) generalized seizure duration of the three stage 5 seizures. Note: the AD durations of the K + TiDB-R group was not analyzed because stimulation artifacts masked the AD recordings. Data are the means ± SEMs and were analyzed by two-way ANOVA for repeated measures followed by Bonferroni's post hoc tests for A and B. One-way ANOVA followed by Bonferroni's post hoc tests was used for C, D and E. n = 9 for each group. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 represent differences from the control group. #p ≤ 0.05, ##p ≤ 0.01 and ###p ≤ 0.001 represent differences compared with the K + TiDB-R group. Abbreviations: ADD, afterdischarge duration; GSD, generalized seizure duration; K + TiDBS, temporally irregular DBS before AK stimuli; K + TiDBS, temporally irregular DBS 5 min after AD; K + TiDBS temporally irregular DBS immediately after AK stimulation.

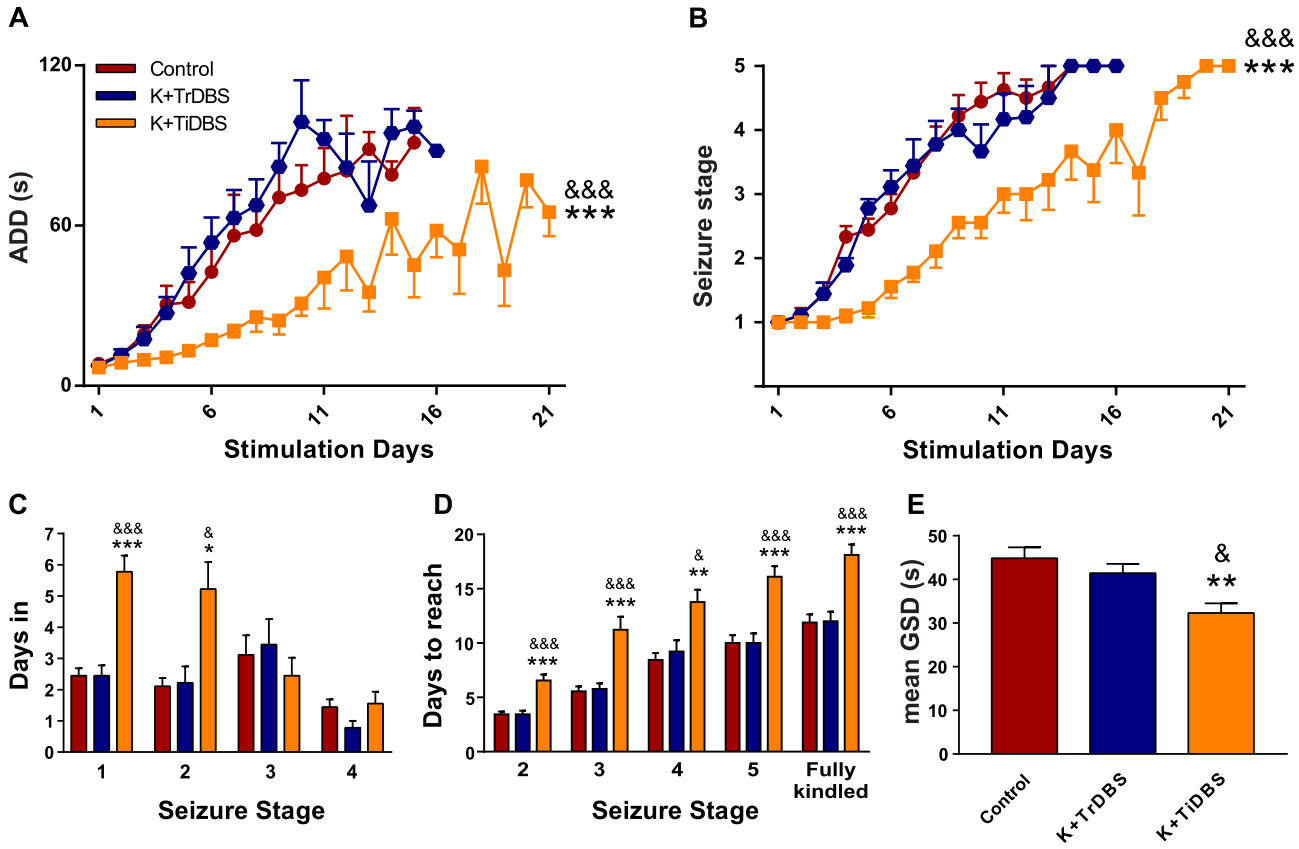

Fig. 5. Comparison of TiDBS and TrDBS. Effects of K + TiDBS and TrDBS on daily ADD (A), seizure stages (B), days of stimulation at each stage, (D) days of stimulation required to reach each stage during kindling acquisition, and (E) generalized seizure duration of the three stage 5 seizures. Data are presented as the means ± SEMs and were analyzed by two-way ANOVA for repeated measures followed by Bonferroni's post hoc tests for A and B and one-way ANOVA followed by Bonferroni's post hoc tests for C to E. n = 9 for each group in every analysis. *p ≤ 0.05, **p ≤ 0.01 and ***p ≤ 0.001 represent differences from the control group. &p ≤ 0.05, &&p ≤ 0.01 and &&&p ≤ 0.001 represent differences from K + TrDBS. K + TrDBS, temporally regular DBS 5 min after AD, the other abbreviations are the same as in Fig. 4.

each seizure stage compared with the control group (Fig. 4A and B). No significant differences were noted when comparing K + TiDBS and TiDBS + K (Fig. 4A and B).

Next, the mean GSD of stage 5 seizures was evaluated. K + TiDBS ($p < 0.001$), TiDBS + K ($p < 0.001$) and, surprisingly, K + TiDBS-R ($p \leq 0.009$) decreased the mean GSD compared to that of the control group (Fig. 4E). No differences were found between K + TiDBS, TiDBS + K and K + TiDBS-R (Fig. 4E). All of the data together confirm that application of TiDBS at the kindling focus delays epileptogenesis and reduces the severity of seizures when it is applied before or after AK stimulation, possibly by interfering with the propagation of ictal activity from the seizure onset zone towards other structures.

Experiment 2. Comparison between TiDBS and TrDBS on kindling epileptogenesis

The effects of K + TiDBS compared to those of the control group are described in Experiment 1. Compared with the K + TrDBS group, K + TiDBS reduced the mean ADD ($p < 0.001$; Fig. 5A), required more days of stimulation for behavioral progression of seizure stages ($p < 0.001$; Fig. 5B), caused rats to remain in stage 1 and 2 longer (stage 1, $p < 0.001$ and stage 2, $p < 0.01$; Fig. 5C), and required a greater number of stimulations to reach each stage as well as the fully kindled state (stages 2, 3, 5 and fully kindled, $p \leq 0.001$; stage 4, $p \leq 0.01$; Fig. 5D). K + TrDBS had no effects on ADD and seizure stage compared to the control group (Fig. 5A and B). Finally, the mean GSD was lower in the K + TiDBS group than in the K + TrDBS group ($p < 0.05$; Fig. 5E), and no differences were found between the K + TrDBS and control groups (Fig. 5E). Although the TiDBS and TrDBS groups had the same mean pulse rate, the protective effects were only present when TiDBS was applied. According to this experiment, the beneficial outcomes obtained from DBS were due to the irregularity of the temporal intervals between pulses and not to other parameters.

Effects of TiDBS on epileptiform propagation

The data obtained showed that the WCCs in the frequency band (1–50 Hz) of each pair of structures was greater in the control rats than in the K + TiDBS and TiDBS + K rats (Fig. 6A–C). The WCC of the right BLA to right MC was greater in the control group than in the K + TiDBS and TiDBS + K groups ($p \leq 0.001$ for both, Fig. 6A). The WCC of the right BLA to left MC was greater in the control group than in the K + TiDBS and TiDBS + K groups ($p \leq 0.001$ and $p \leq 0.01$, respectively, Fig. 6B). The WCC of the right MC to left MC was greater in the control than in the K + TiDBS and TiDBS + K ($p \leq 0.001$ and $p \leq 0.01$, respectively, Fig. 6C). No differences were observed between the K + TiDBS and TiDBS + K groups. It is important to note that the WCC increased progressively in the control group, while it remained almost constant in the K + TiDBS and TiDBS + K groups. These results demonstrate that the propagation of the epileptiform activity from the seizure onset zone to the ipsi- and contralateral hemispheres and between them was delayed by TiDBS.

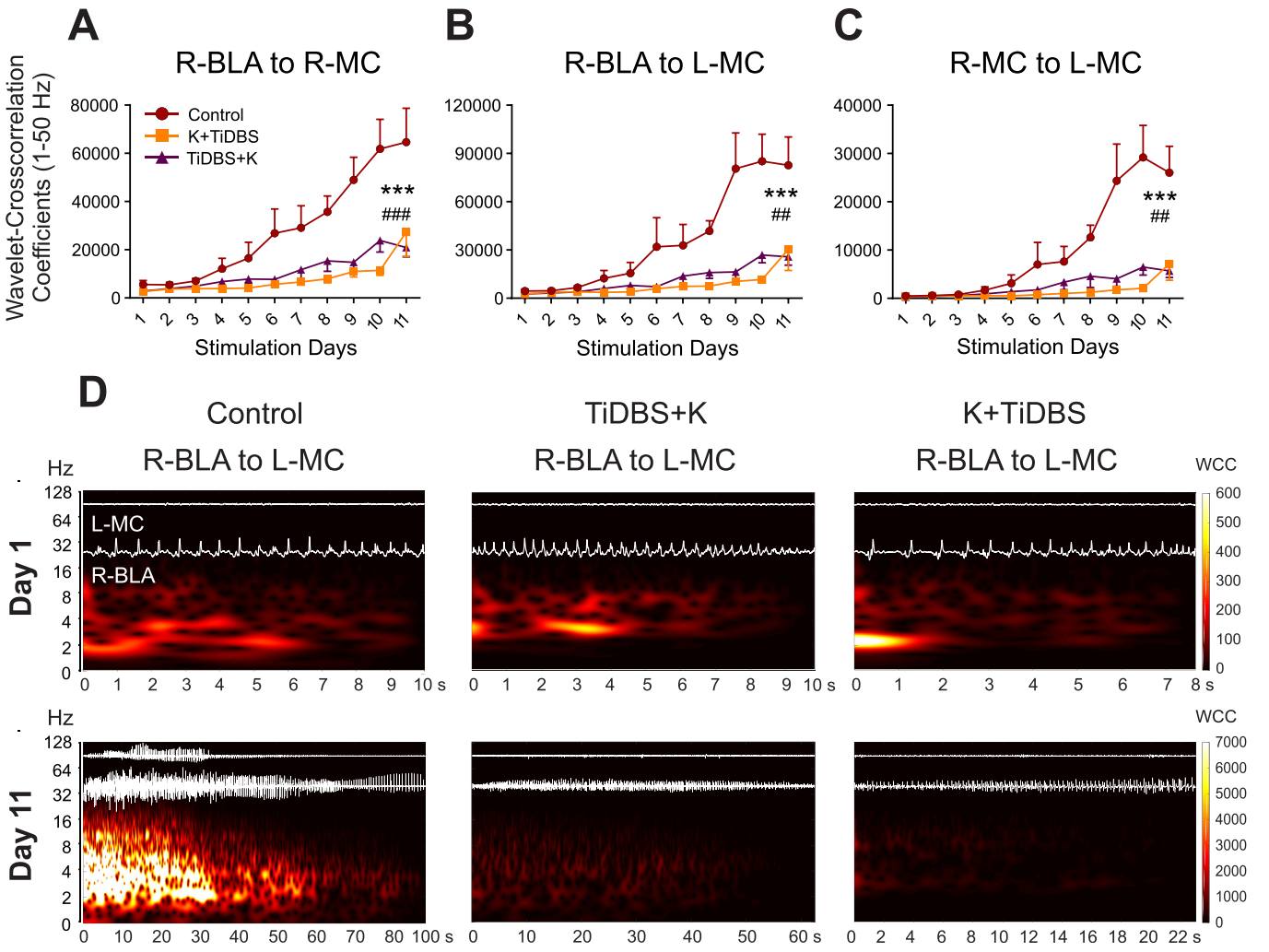

Fig. 6. Effects of TiDBS on epileptiform propagation measured using wavelet cross-correlation analysis. Quantification of the daily WCC (1–50 Hz) per group of the R-BLA to R-MC (A), R-BLA to L-MC (B) and R-MC to L-MC (C). Notice that the WCC of TiDBS + K and K + TiDBS is less than that of the control group between all pairs of structures. (D) Representative traces and WCC heat maps of the propagation from the R-BLA to L-MC of an AD at day 1 and day 11 of AK stimulation in the control, TiDBS + K and K + TiDBS groups. Data are presented as the means ± SEMs and were analyzed by two-way ANOVA for repeated measures followed by Bonferroni's post hoc tests. n = 9 for each group. Control versus K + TiDBS (***$p \leq 0.001$) and control versus TiDBS + K (##+$p \leq 0.01$, ###$p \leq 0.001$). Abbreviations: L-MC, left motor cortex; R-MC right motor cortex; R-BLA, right basolateral amygdala; WCC, wavelet cross-correlation coefficients.

Relationship between IED and TiDBS

The analysis of the IEDs showed no significant differences between the means of the IED ratios, but a slight increasing trend was observed in the IED ratios of the K + TiDBS group versus the control group (Fig. 7A). However, a negative correlation was found between the mean GSD and the IED ratios of rats in the K + TiDBS ($r = -0.822$, $n = 9$, $p = 0.012$) (Fig. 7C) and TiBDS + K ($r = -0.720$, $n = 9$, $p = 0.029$) (Fig. 7D) groups but not for the control group ($r = -0.085$, $n = 9$, $p = 0.828$) (Fig. 7B). This suggests that IEDs are associated with the delivery of TiDBS and the severity of seizures.

Discussion

Electrical stimulation of specific brain areas to treat pharmacoresistant epilepsy has been studied since the 20th century [27]; however, full control of seizures and epileptogenic processes has not yet been achieved. Epileptogenesis is the process by which a healthy brain turns into an epileptic brain [28]; thus, targeting this critical period is important to prevent the establishment of epilepsy [29]. During ictogenesis and epileptogenesis, specific microcircuits outside the seizure onset zone are recruited and generate an epileptic network [30,31]. The present study demonstrates that TiDBS delayed epileptogenesis by interfering with the propagation of epileptiform activity when applied during the interictal period. Thus, it might be possible that this delay modifies microcircuit recruitment.

It is known that the BLA sends direct efferents to the hippocampus, striatum and frontal cortex [32,33], which are possible target structures for epileptiform propagation. Indeed, correlated neural firing has been found in those structures when an AD is elicited [34]. Here, we specifically found a greater difference in the WCC from the BLA to left MC when comparing TiDBS rats to control rats. This suggests that pathways from the BLA to the contralateral hemisphere are more affected than those to the ipsilateral hemisphere. IEDs are generated by the synchronous firing of a cluster of neurons [35] and are well documented during AK epileptogenesis. Indeed, it has been shown that IEDs correlate with decreased seizure susceptibility in vitro [36–38] and in vivo [39]. Here, we found a similar association between the IED ratio and seizure

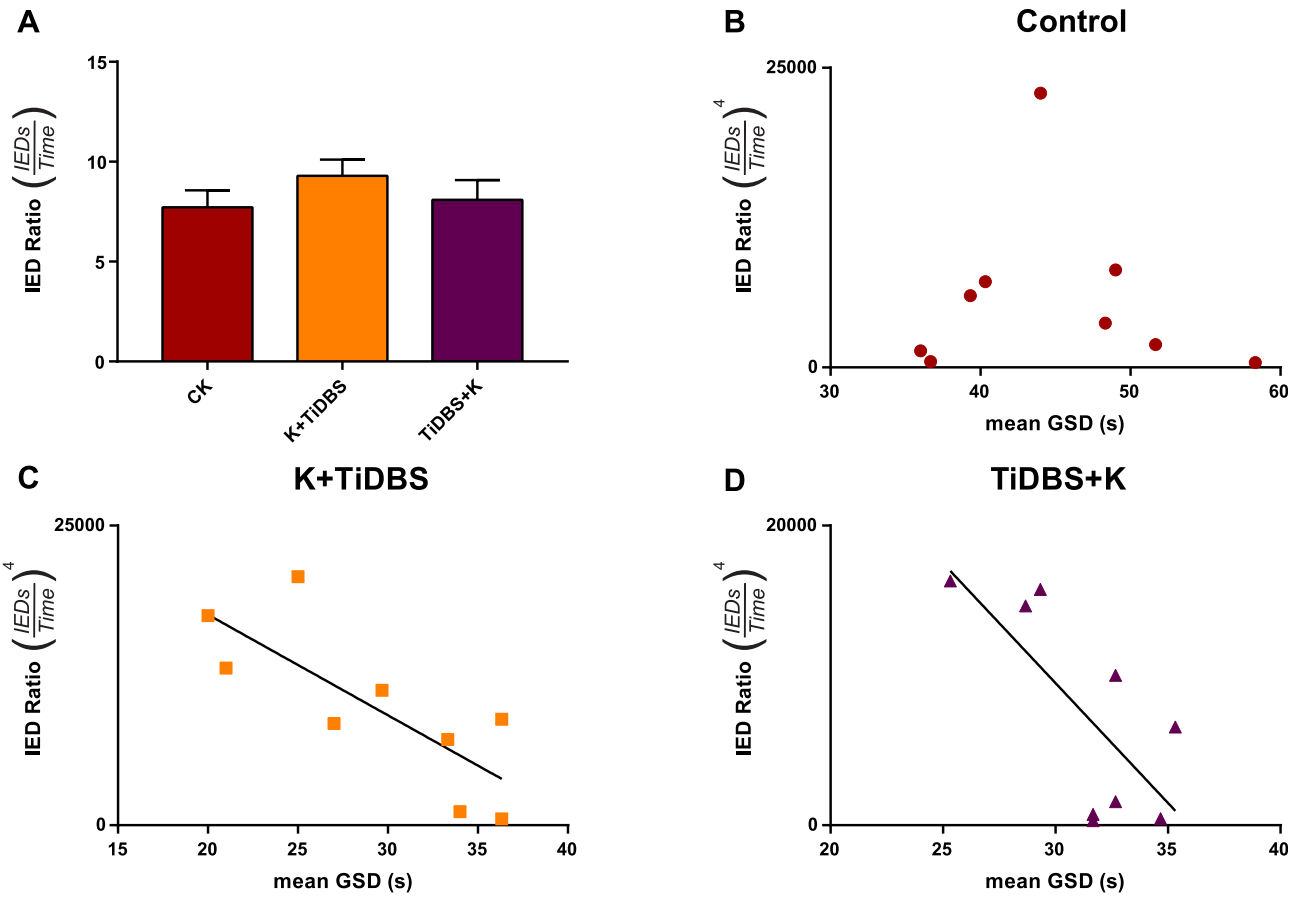

Fig. 7. Effects of TiDBS on IEDs. (A) Quantification of the IED ratio 5 min after AD. No differences were found; however, a slight trend in the K + TiDBS group was observed. Correlation analysis between the transformed IED ratio and the mean GSD in control rats (B), K + TiDBS rats (C) and TiDBS + K rats (D). An association was found only in the K + TiDBS and TiDBS + K groups. Data are presented as the means ± SEMs and were analyzed by one-way ANOVA followed by Bonferroni's post hoc tests for panel A. Data are presented as scatter points for each rat and analyzed by Pearson's correlation coefficient test for panels B to D. n = 9 for each group. r = −0.085, n = 9, p = 0.828 in B; r = −0.822, n = 9, p = 0.012 in C; and r = −0.720, n = 9, p = 0.029 in D. Abbreviations: IED, interictal epileptiform discharge.

severity of rats that received TiDBS; therefore, we suggest that TiDBS may favor the appearance of IEDs.

It has been recently documented that IEDs can facilitate or prevent seizures depending on the state of the brain [40]. If the underlying neuronal network is stable—for example, when a seizure ends—IEDs have antiseizure properties. If the neuronal network is losing resilience, then IEDs act as destabilizing perturbations that lead to seizure generation. This is supported by the fact that the induction of IEDs suppresses seizures in an in vitro model [41] and is in accordance with the current notion that synchronicity facilitates the termination of ictal activity instead of provoking it [15]. This could explain why TiDBS applied after AD is effective; however, this may seem insufficient to explain the effect obtained when TiBDS is applied before the AD. It is true that when a network is approaching a seizure, it becomes fragile and loses resilience; however, this is not the case in the classic kindling model where the seizures are provoked. Thus, before an AD, the network is still in a stable state in which IEDs can exert their protective effects.

On the other hand, when TiDBS was applied during the AD, it only shortened the GSD but had no other effects on epileptogenesis. During stages 4 and 5, rats presented unusual behaviors such as wild running, a behavior not typically observed in AK rats [18], and did not spend much time in a rearing position. Wild running is common to other animal models in response to elevated sound stimuli [42,43] and is associated with brainstem activation [44]. We believe there might be two possibilities underlying this observation: the first is that the stimulation favors recruitment of the brainstem through the central amygdala; the other possibility is that it may be promoting general arousal [45] by activating the striatum due to the direct afferents from the BLA [32].

The question that then arises is: why is TiDBS effective in our study while TrDBS is not?

The firing rate of neuronal spiking in vitro and in vivo significantly varies over extended timescales (from milliseconds up to several hours) and appears in spontaneous as well as evoked activity upon repeated stimulus presentation [46]. It has been documented that irregular patterns of electrical stimulation increase the reliability or regularity of neuronal firing in single neurons [47,48] and result in the generation of synchronous firing in population neurons [49]. In contrast, when sequences of temporally regular pulses are applied, the neural response becomes intermittent and irregular (unreliable) [48,49] and generates asynchronous firing in target neurons [50–52]. Therefore, TiDBS might provoke anti-epileptogenic effects through the generation of synchronous firing of the BLA, locking itself to input fluctuations and facilitating IEDs, while TrDBS produces highly variable responses that are not effective in interfering with epileptogenesis.

This study suggests that implementing temporally irregular patterns in stimulation trains could be a suitable strategy against epilepsy and other neurological disorders, but it also provides researchers and clinicians with a new set of parameters that can be modified to optimize DBS.

Conclusion

In the present study, we showed that TiDBS delivered during the interictal period delayed epileptogenesis by interfering with epileptiform propagation from the epileptogenic focus to the ipsi- and contralateral hemispheres. Thus, TiDBS could be a preventive approach against epilepsy.

Conflicts of interest

The authors report no conflicts of interest with this work.

Acknowledgments

This study was partially supported by the Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico, project NC123240.1. We thank Psych. Diego Flores-Escobar and Mr. Edgar Urbina-Trejo for his technical assistance as well as Dra. Ana Paula Rivera-García for proof-reading this manuscript.

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