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Deep brain stimulation probing performance is enhanced by pairing stimulus with epileptic seizure

Daniel de Castro Medeiros [a,b], Laio Bastos Paiva Raspante [a], Flávio Afonso Gonçalves Mourão [a,b], Vinícius Rezende Carvalho [a,b], Eduardo Mazoni Andrade Marçal Mendes [b], Márcio Flávio Dutra Moraes [a,b,*]

[a] Núcleo de Neurociências, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas - Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
[b] Centro de Tecnologia e Pesquisa em Magneto Ressonância, Programa de Pós-Graduação em Engenharia Elétrica - Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

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ABSTRACT

The unpredictability of spontaneous and recurrent seizures significantly impairs the quality of life of patients with epilepsy. Probing neural network excitability with deep brain electrical stimulation (DBS) has shown promising results predicting pathological shifts in brain states. This work presents a proof-of-principal that active electroencephalographic (EEG) probing, as a seizure predictive tool, is enhanced by pairing DBS and the electrographic seizure itself. The ictogenic model used consisted of inducing seizures by continuous intravenous infusion of pentylenetetrazol (PTZ — 2.5 mg/ml/min) while a probing DBS was delivered to the thalamus (TH) or amygdaloid complex to detect changes prior to seizure onset. Cortical electrophysiological recordings were performed before, during, and after PTZ infusion. Thalamic DBS probing, but not amygdaloid, was able to predict seizure onset without any observable proconvulsant effects. However, previously pairing amygdaloid DBS and epileptic polyspike discharges (day-1) elicited distinct preictal cortically recorded evoked response (CRER) (day-2) when compared with control groups that received the same amount of electrical pulses at different moments of the ictogenic progress at day-1. In conclusion, our results have demonstrated that the pairing strategy potentiated the detection of an altered brain state prior to the seizure onset. The EEG probing enhancement method opens many possibilities for both diagnosis and treatment of epilepsy.

1. Introduction

Epilepsy is a common neurological disorder that affects approximately 1% of the world population, characterized by the occurrence of unpredictable and recurrent seizure episodes [1]. The unforeseen seizures are a major concern for all patients, particularly for those with pharmacoresistant epilepsy [2], an alarming one-fourth of the total, significantly impacting on the patient's quality of life [3]. Reliable methods for seizure prediction would most certainly improve the welfare of patients with epilepsy [4,5] while also having potential applications in closed-loop therapeutic strategies [6].

In general terms, seizure prediction systems aim to detect the shift to an altered brain state prior to the onset of epileptiform discharges — via electroencephalographic (EEG) signal analysis [7]. Despite recent progress, most methods based solely on EEG analytical processing still lack sufficient reliability [8]. New strategies centered on deep brain electrical stimulation (DBS) and its evoked neural responses have been shown to have promising seizure predictive capability [9,10]. In other words, stimuli have been used to probe the excitability level of neural circuits [11], considered an important preictal state marker [12,13], without relying on the eventuality of passively recording a preictal marker.

A complicating factor inherent to the probing/evoked response approach is the optimal choice for the stimulation and recording sites, which are specific to each epileptic condition. Such a choice must aim to safely evoke a detectable preictal response, without increasing the likelihood of seizure occurrence. Within the epileptic seizure types, despite the peculiarities of each condition, a common characteristic is the abnormal synchronization of distributed neural circuits during the ictal discharges, particularly at the final stages of the epileptiform activity [14]. In addition, seizure repetition has been shown to induce plastic changes that directly impact seizure spread and network recruitment, eventually increasing the severity of the epileptic condition itself ([15,16] and for review see [17,18]). A possible explanation is that the pathological synchronicity of neuronal firing during seizures may strengthen the connections among multiple circuits [19,20], per Hebb's postulate "neurons that fire together, wire together" [21,22]. According to the Hebbian plasticity rule, the synaptic network efficacy is enhanced by the activity of the neurons temporally correlated, crucial to long-term potentiation [23]. Within this context, we hypothesized that if a

* Corresponding author at: Núcleo de Neurociências, Departamento de Fisiologia e Biofísica, Instituto de Ciências Biológicas - Universidade Federal de Minas Gerais, Av. Antônio Carlos, 6627 - CEP 31270-901- Campus Pampulha, Belo Horizonte, MG, Brazil.
E-mail address: mfdm@icb.ufmg.br (M.F.D. Moraes).

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stimulus is applied during the epileptiform activity, similar plastic processes would increase the connectivity between the stimulated area and seizure-related networks. In fact, because of the nature of its polysynaptic circuitry, the network association would most likely encompass the focus and the spreading pathways [24], which would theoretically improve neural circuitry probing during the ictogenesis process.

This work aimed to enhance the probing strategy for seizure prediction by previously pairing DBS to ictal activity in an acute epileptiform activity model. In more detail, our protocol is designed to elicit a cortically recorded evoked response (CRER), triggered by an amygdala (AMY) electric stimulation, only if stimuli were previously paired (and unpaired for the control groups) with pentylenetetrazol-induced (PTZ-induced) seizure. The AMY was chosen as the pairing electric stimulation site not only because of its extensive and reciprocal connections to and from cortical regions but also for its highly plastic neuronal substrates [25]. In addition, DBS probing was also investigated targeting the thalamus (anterior nucleus of the thalamus (TH)). Thalamic electrical stimulation has long been used in epilepsy research with promising results at seizure suppression [26]. In fact, long-term follow-up studies have demonstrated sustained efficacy in the reduction of seizures by using DBS in the anterior nucleus of the TH [27,28]. Furthermore, the thalamocortical monosynaptic link [29] is an interesting neural framework for studying evoked potential responses prior to ictal discharges. Although the PTZ model is not typically used for the seizure prediction research, it, nevertheless, allows for a controlled and gradual ictogenesis progress, quite suitable for this study. The results show that TH, but not AMY, DBS probing elicited detectable cortical responses prior seizure onset. Nevertheless, by previously pairing the AMY electrical stimulation and the epileptiform discharges (day-1), a CRER was detected at the preictal period (day-2), without increasing seizure susceptibility. Our work demonstrates that probing epileptogenic circuits for seizure prediction is potentiated and made more efficient by previously pairing the probing stimuli to the ictogenic process.

2. Experimental procedures

2.1. Subjects

Male Wistar rats (weighing 300–320 g), supplied by the Biotério do Instituto de Ciências Biológicas 2 (BICBIO 2) vivarium, were housed under controlled environmental conditions (22 ± 2 °C), with a 12:12-hour light–dark cycle and free access to food and water. All experiments have been approved by the Ethical Committee for the Use of Animals (CEUA) – Universidade Federal de Minas Gerais – under license number 112/2014. The CEUA directives comply with National Institutes of Health (NIH) guidelines for the care and use of animals in research.

2.2. Stereotaxic surgery

Rats were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) administered intraperitoneally and positioned in a stereotaxic frame (Stoelting Co., Wood Dale, IL, USA). The scalp was injected with subcutaneous anesthesia (lidocaine, 5 mg/kg) before incision. Bipolar electrodes (2 mm tip separation dipole), made of a twisted pair of stainless-steel Teflon-coated wires (Model 791400, A-M Systems Inc., Carlsborg, WA, USA), were surgically implanted in the right AMY (anteroposterior (AP): −2.8 referenced from the bregma, mediolateral (ML): −5.0, dorsoventral (DV): −7.2) or right TH (anterior nucleus of TH — AP: −1.4 referenced from the bregma, ML: −1.0, DV: −5.2) for the DBS. In addition, surface electrode microscrews were positioned over both parietal cortices (AP: −1.0 referenced from the lambda, ML: ±2.0) for local field potential (LFP) recordings. The electrodes were then soldered to a connector (registered jack - RJ 11-6 pins), which in turn was fixed to the rat's skull with dental acrylic cement.

2.3. DBS and PTZ infusion

The electric stimulus consisted of a monophasic square pulse (0.1 ms pulse width and 0.8 mA intensity — Fig. 1A) delivered at 0.5 Hz by an isolated constant current stimulator (Digitimer® DS3 Constant Current Stimulator). The DBS protocol was designed to minimize charge delivery by means of short pulse duration and a low repetition rate (0.5 Hz). In addition, considering the limited number of pulses used (approximately 200 pulses on Protocol-one and 350 on Protocol-two), no significant tissue damage [10] was observed in histology at the stimulation site.

The PTZ infusion (10 mg/ml PTZ Sigma-Aldrich diluted in saline 0.9%) was performed through the cannulated medial tail vein (BD Angiocath Catheter I.V.; 24GA × 0.75IN — 0.7 × 19 mm) at a rate of 2.5 mg/ml/min (infusion pump KDS100 — KD Scientific). The PTZ infusion was interrupted at the beginning of the EEG epileptic discharges, for all protocols.

2.4. Electrophysiological recordings and analysis

All LFP recordings can be divided into three periods: Basal period (no external intervention — 120 s), electrical stimulation template (EST) period (application of sixty electrical stimulation pulses — 120 s), and the experimental period which differed according to each protocol and each group. The LFP signal from parietal cortices was amplified (1000× gain) and filtered (1 Hz High-pass, 500 Hz Low-pass) by a signal conditioner unit (Aisha4-Kananda® Ltd.). A trigger signal from the electrical pulses was also recorded on a different channel (no filters nor amplification) to obtain the exact stimulus timestamp. Data were sampled at 1 kHz and stored on a computer hard disk for offline analyses.

The LFP analysis was performed by employing custom routines and functions from the MATLAB® 7.12 R2010 (MathWorks) package. The stimulus trigger signal was used to segment the LFP recordings in a sequence of 2-second time epochs, before (500 ms) and after (1500 ms) the electrical stimulation. A template derived from the average of all 2-second epochs during the EST period was calculated per animal (Fig. 1C) to establish a baseline that would include artifact contaminants. Each 2-second epoch from the experimental period was then subtracted from the template in order to extract the electrical artifact from the LFP signal (Fig. 1D). Next, the normalized energy, calculated as the LFP power after deep brain electrical stimulation (A_{DBS} = 10 to 270 ms referenced to stimulus timestamp) normalized by the baseline power before the DBS (B_{DBS} = −250 to 0 ms referenced to stimulus timestamp), was quantified for every 2-second epoch. In addition, the spectral entropy of A_{DBS} was analyzed throughout the ictogenic process by:

$$E = \sum f - p(f)^2 \ \log\left(\mathrm{p}(f)^2\right)$$

where $p(f)$ are fast Fourier transform (FFT) coefficients of frequency f [30]. The time evolution throughout the ictogenic process of the spectral entropy was calculated at the gamma frequency band (30–80 Hz; Fig. 4E–G) [31] and also for different frequency bands, 8 Hz half-band bin size (Fig. 4D). The spectral entropy quantifies the signal regularity/complexity by the measure of the frequency spectrum distribution [32]. In biological terms, spectral entropy indicates the organization level among distinct neural circuits, and it was used in this work to evaluate the oscillatory synchrony induced by the pairing-DBS prior ictal discharges.

The electrographic seizure activity onset, named time zero (Tzero), was set at the beginning of observed continuous (minimum of 3 s duration) high amplitude epileptiform polyspike discharges. The time period from the beginning of the PTZ infusion and electrographic seizure activity onset was named seizure latency. The duration of the high amplitude

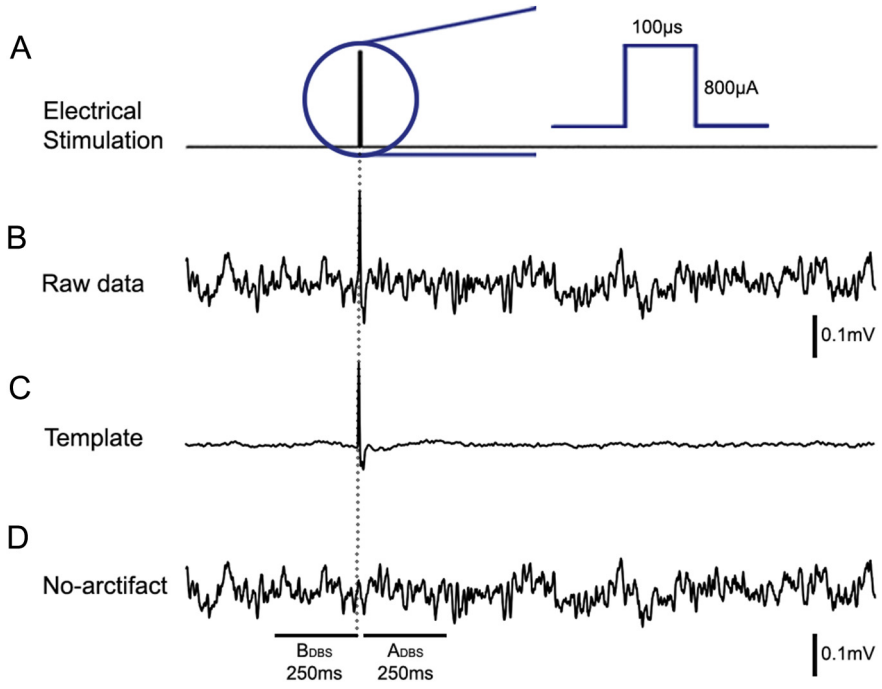

Fig. 1. Sequential steps for removing electrical stimulus artifact. A: Electrical stimulation trigger recording and the pulse duration and intensity. B: raw data demonstrating the electrical stimulation artifact. C: typical 2 s template calculated from the average of EST period 60 electrical pulses (120 s). D: Artifact removed from the signal. The normalized energy (A_{DBS}/B_{DBS} power ratio) was then calculated for each 2-second signal with no artifact.

polyspike epileptiform activity was termed seizure duration. Both seizure latency and duration were used to quantify seizure severity.

2.5. Experimental design

This work was divided into two main protocols, with distinct objectives, which used separate sets of animals. While Protocol-one aimed to verify the preictal cortical response evoked by applying the DBS to two different structures (TH and AMY) the goal of Protocol-two was to test if DBS previously paired to epileptiform activity would potentiate the effect of stimulation as a seizure predictor. All animals were subjected to the same Basal period (no external intervention — 120 s) and EST period before initiating the protocol-specific experimental procedure.

2.6. Protocol-one

Rats were divided into two main groups according to the electrode-position, TH, or AMY. Both groups were further separated into three subgroups, according to the experimental period: PTZ infusion (PTZ with no DBS — PTZ-noDBS group, TH n = 6; AMY n = 7), electrically stimulated (DBS with no PTZ — DBS-noPTZ group, TH n = 6; AMY n = 5), and PTZ infusion associated with DBS (PTZ + DBS group, TH n = 9; AMY n = 9).

2.7. Protocol-two

Since the AMY stimulation group from Protocol-one presented the weakest response as a surrogate marker for seizure detection, Protocol-two used only AMY-implanted animals. Protocol-two animals were submitted to a two-day experimental procedure.

2.8. Day-1

All rats received PTZ infusion until the electrographic seizure activity onset (experimental period – Fig. 3A – gray upper LFP trace). During the first day, animals were divided into three groups according to DBS (120 s) and seizure activity pairing (Fig. 3A): not paired with DBS presented at the beginning of PTZ infusion (B-ES group — before epileptic seizure, n = 6); DBS paired with seizure activity (ES group — epileptic seizure, n = 7); not paired with DBS presented 300 s after postictal activity (A-ES group — after the epileptic seizure, n = 6). For the ES group, the DBS started at the first generalized and robust muscle twitch accompanied by isolated individual spike on the LFP signal [33], usually occurring 20 s before the electrographic seizure activity onset. It is important to highlight that all animals from ES group received stimulation during the whole time electrographic seizure activity was being observed, i.e., the behavioral and electrographic marker used ensured that seizure activity fell inside the 120-second time window stimulation period. Immediately after the day-1 session, all animals were returned to their home cage under controlled environmental conditions (22 ± 2 °C — 12:12-hour light–dark cycle) and free access to food and water.

2.9. Day-2

Twenty-four hours after the day-1 session, PTZ infusion associated with DBS was applied to all animals until the electrographic seizure activity onset (experimental period – Fig. 3B – blue lower LFP trace). This procedure aimed to verify how effectively the CRER changed before seizure onset, i.e., how stimulation effectively worked as a seizure predictor.

2.10. Histology

Postmortem verification of electrode placement was initiated immediately after the last LFP recording. Animals were anesthetized (Urethane 14% w/v; 10 ml/kg), and a current of 0.2 mA was applied to the electrode for 5 s, allowing it to mark the targeted structure for histological confirmation. Animals were perfused with saline and, subsequently, with 4% paraformaldehyde (PFA). The brain was removed and placed in a 4% PFA solution overnight and then moved to a solution of 30% sucrose diluted in phosphate-buffered saline. Tissue was maintained at 4 °C for a minimum of 3 days. For the histological identification of electrode position, 50 µm coronal brain slices were obtained using a cryostat (Cryostat 300 e ANCAP Ltd.) and stained with neutral red (2%). Only data from

animals with the correct implantation of the electrodes were included in the subsequent statistical analysis.

2.11. Statistical analysis

All data were analyzed using GraphPad Prism 5® (GraphPad Software). Results were plotted as the mean ± standard error of the mean and considered significant if $p < 0.05$. The Kolmogorov–Smirnov test was used to confirm the normal distribution of data. The statistical comparisons were performed by Student's t-test, one-way, or 2-way analysis of variance (ANOVA) followed by a post hoc test.

3. Results

3.1. Electrical stimulation during PTZ infusion did not change seizure severity

Neither the TH nor the AMY electrical stimulation altered PTZ-induced seizure severity; recorded features: duration and latency (PTZ threshold for seizure onset — g/kg). As shown in Fig. 2, no statistical difference was found between the PTZ-noDBS and PTZ + DBS groups (unpaired Student's t-test) for seizure duration [TH $t(10) = 0.8$, $p = 0.4$ — Fig. 2C; AMY $t(14) = 0.27$, $p = 0.7$ Fig. 2G] or seizure latency [TH $t(13) = 1.2$, $p = 0.2$ — Fig. 2B; AMY $t(14) = 1.6$, $p = 0.1$ — Fig. 2F].

3.2. AMY probing stimulation failed to predict seizure onset

The TH or AMY electrical stimulation did not produce similar preictal CRER, as demonstrated by representative PTZ + DBS animal from both groups at Fig. 2I–J and depicted in the normalized energy value dynamics throughout the 120 s before the seizure onset (5-element moving average; Fig. 2D and H respectively). The TH PTZ + DBS group showed a statistically significant increase of normalized energy at least 10 s before the seizure onset when compared with PTZ-noDBS and DBS-noPTZ groups [Fig. 2D — Interaction Time × Groups: $F(118,1062) = 2.5$, $p = 0.0001$; Time: $F(59,1062) = 1.3$, $p = 0.04$; Groups: $F(2,18) = 17.3$, $p = 0.0001$; Two-way ANOVA — Bonferroni's post hoc test $p < 0.05$ for −10 s to Tzero]. In contrast, there was no evident change in the cortical-recorded evoked response in the AMY groups during the preictal period, as can be seen in Fig. 2H–J. In fact, the AMY PTZ + DBS group demonstrated no sustained change on normalized energy value when compared with PTZ-noDBS and DBS-noPTZ groups [Interaction Time × Groups: $F(118,1062) = 0.6$, $p = 0.9$; Time: $F(59,1062) = 0.4$, $p = 0.9$; Groups: $F(2,18) = 7$, $p = 0.005$;

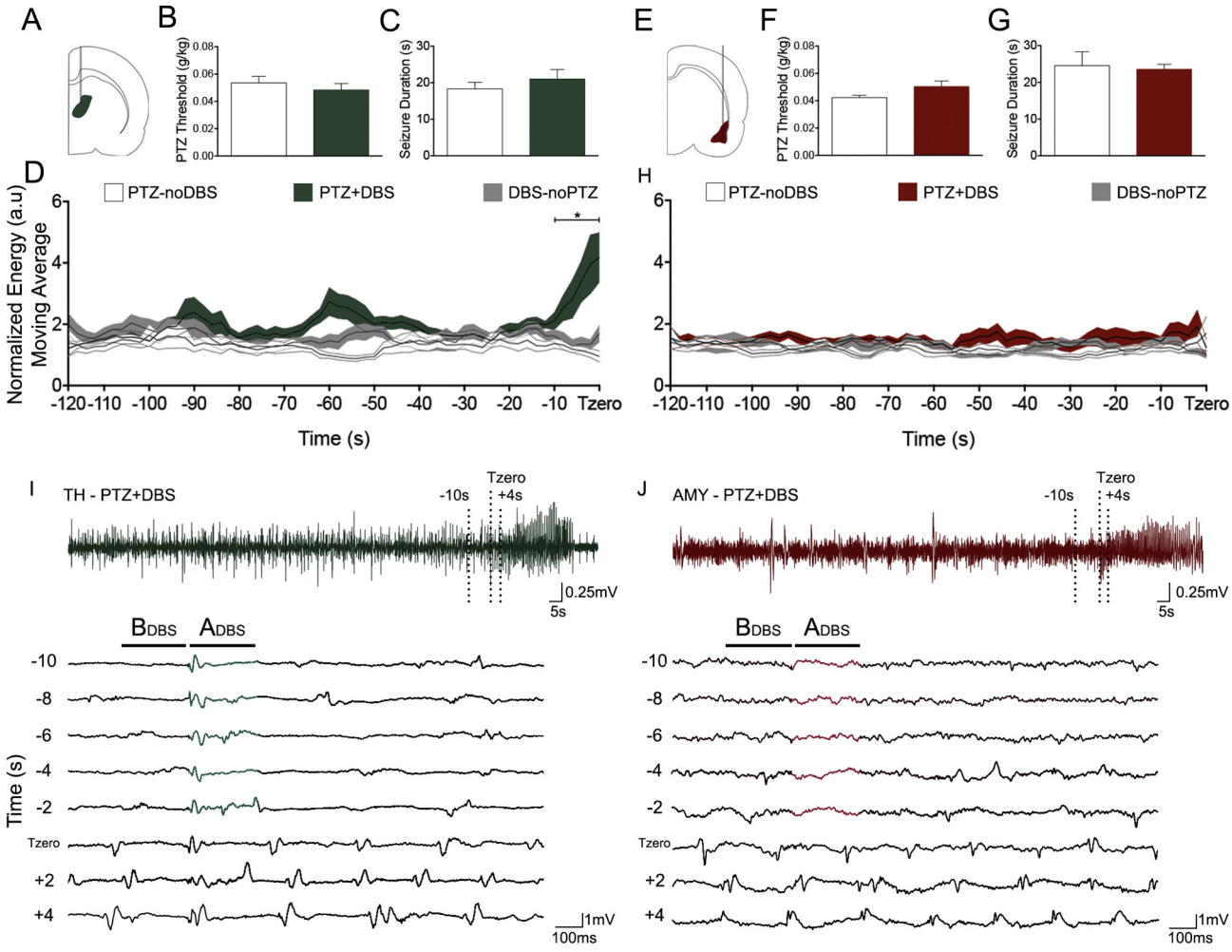

Fig. 2. The TH (left) and AMY (right) probing strategy elicited distinct outcomes. A–E: Representation of the bipolar electrodes placement. B–F: Seizure latency presented as PTZ threshold. C–G: Seizure duration. D–H: Normalized energy (moving average five elements) during the PTZ ictogenic progress. I–J: Representative LFP signals from TH and AMY PTZ + DBS groups. The bottom panels show the time series that highlight the period preceding seizure activity (−10s to +4 s referenced by ictal onset) in a sequence of 2-second epochs (−0.5 to 1.5 s of the DBS trigger). * denotes $p < 0.05$ for group comparisons.

Two-way ANOVA — Bonferroni's post hoc test $p < 0.05$ at −74 s and at −118 s for PTZ + DBS and DBS-noPTZ].

3.3. No evidence for a kindling-like process occurring in Protocol-two

Different neural excitability features (Fig. 3) were quantified to address the effect of a possible kindling phenomenon caused by two consecutive seizure inductions at a 24-hour interval. To that affect, the mean power (V^2) from the Basal period (Fig. 3B) presented no statistically significant difference between day-1 and day-2 [**Mean Basal Power**: Interaction Groups × Days: $F(2,16) = 1.5$, $p = 0.2$; Groups: $F(2,16) = 0.2$, $p = 0.7$; Time: $F(1,16) = 3.3$, $p = 0.08$; Two-way ANOVA — Bonferroni's post hoc test $p > 0.05$]. In addition, seizure severity indicators, such as ictal discharge mean power (Fig. 3E), latency (Fig. 3F), and duration (Fig. 3G), also did not differ between day-1 and day-2 [**Seizure Latency**: Interaction Groups × Days: $F(2,16) = 0.03$, $p = 0.9$; Groups: $F(2,16) = 3.5$, $p = 0.053$; Time: $F(1,16) = 0.5$, $p = 0.4$; **Seizure Duration**: Interaction Groups × Days: $F(2,16) = 0.1$, $p = 0.8$; Groups: $F(2,16) = 1.6$, $p = 0.2$; Time: $F(1,16) = 0.02$, $p = 0.8$; **Seizure Energy**: Interaction Groups × Days: $F(2,16) = 0.03$, $p = 0.9$; Groups: $F(2,16) = 0.7$, $p = 0.4$; Time: $F(1,16) = 5.5$, $p = 0.03$; Two-way ANOVA — Bonferroni's post hoc test $p > 0.05$].

In fact, the only baseline change observed between day-1 and day-2 was for the B-ES group at the EST period analysis (mean normalized energy — Fig. 3C). In contrast with the A-ES and ES groups, the B-ES group had a significantly reduced mean normalized energy at day-2 when

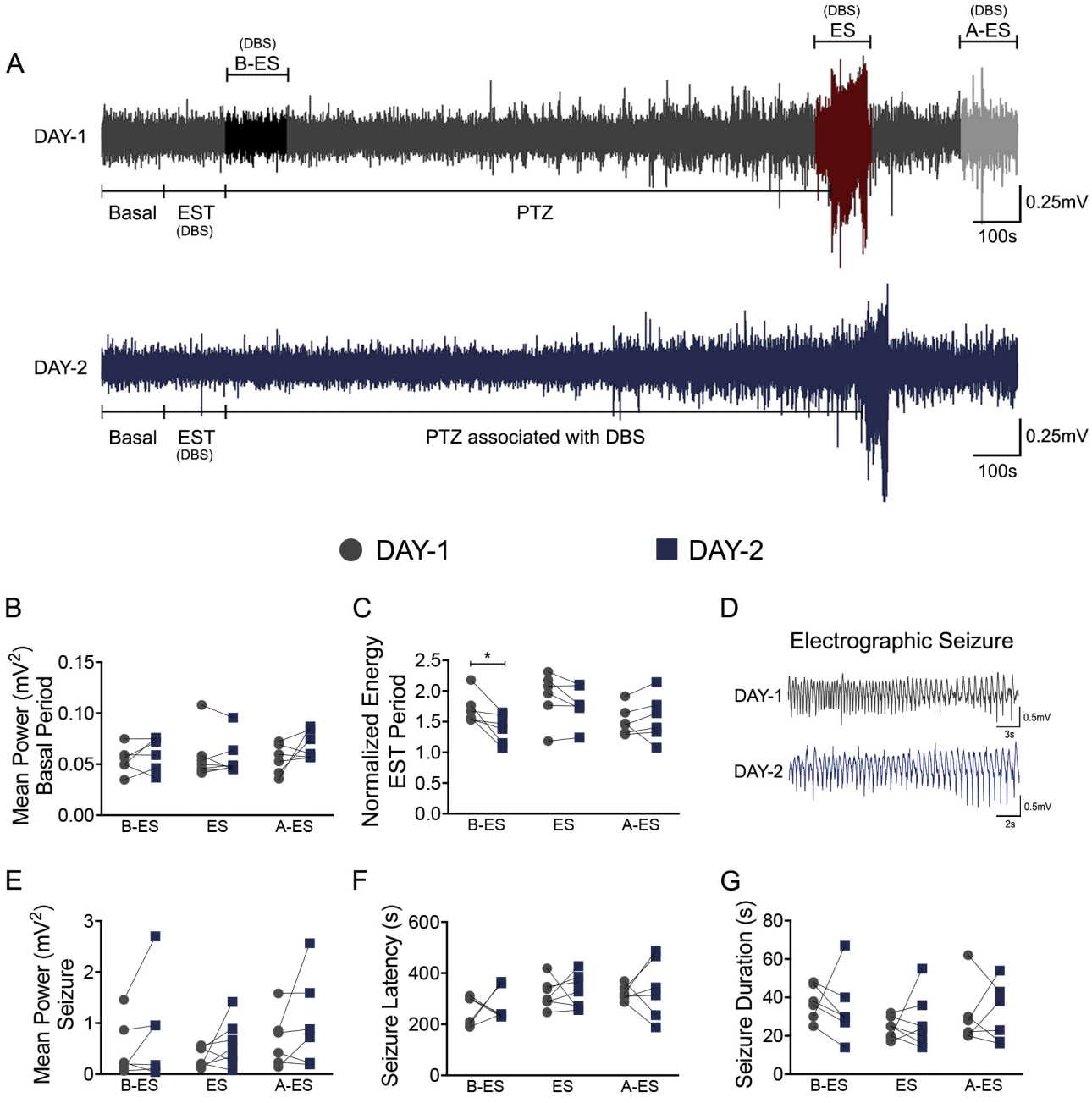

Fig. 3. Day-1 and -2 analysis. A: Experimental design, denoting a typical LFP recording from day-1 (upper gray trace) and day-2 (lower blue LFP). The different position of DBS (120 s) within the PTZ-induced seizure progress is highlighted for each group. B: Basal period mean power (mV^2) comparing day-1 with day-2. C: Mean normalized energy value at EST period for day-1 to -2. D: Typical electrographic polyspike discharge at day-1 to -2 (gray and blue LFP, respectively). E: Electrographic seizure mean power (mV^2). F: Latency for electrographic seizure onset at day-1 to -2. G: Duration of the epileptic polyspike discharges. * denotes $p < 0.05$ for group comparisons.

compared with day-1 [**EST period**: Interaction Groups × Days: $F(2,15) = 4.1$, $p = 0.03$; Groups: $F(2,15) = 2.2$, $p = 0.14$; Time: $F(1,15) = 6.6$, $p = 0.02$; Two-way ANOVA — Bonferroni's post hoc test $p < 0.05$ for B-ES group]. This apparently paradoxical reduction in excitability will be addressed in the Discussion section.

3.4. Paired AMY–seizure at day-1 enhanced the probing stimulation response for subsequent seizure at day-2

By pairing AMY stimulation with seizure activity during day-1, i.e., the ES group, the normalized energy at day-2 significantly increased before seizure onset when compared with the A-ES and B-ES groups (Fig. 4). The specific comparisons between groups from Protocol-two are depicted in Fig. 4B [**Normalized energy**: Interaction Time × Groups: $F(118,944) = 2.0$, $p < 0.0001$; Time: $F(59,944) = 6.1$, $p < 0.0001$; Groups: $F(2,16) = 0.5$; $p = 0.5$ — Two-way ANOVA — Bonferroni's post hoc test $p < 0.05$ for −6 to Tzero for ES group and A-ES group and −4 to Tzero for ES group and B-ES group] and Fig. 4C [**Mean normalized energy** from −6 to Tzero: one-way ANOVA $F(2,16) = 8.9$, $p = 0.002$, Tukey's post hoc test $p < 0.05$]. In fact, only the ES group showed a significant change in spectral entropy prior to seizure onset at day-2 (Fig. 4D–G).

4. Discussion

This work presents a proof-of-principal that active EEG probing, as a seizure predictive tool [34], may be enhanced by previously pairing DBS and the electrographic seizure itself (Fig. 4). Our results show that, unlike thalamic stimulation (Fig. 2D–I), AMY stimulation at day-1 failed to present the dynamical changes that would render it as potentially useful for seizure prediction (Fig. 2H–J). Nevertheless, after the pairing protocol (Fig. 3A), the ES group showed a gradual and significant change throughout the ictogenic process (Fig. 4B). Altogether, data suggest that by pairing DBS with epileptiform activity during day-1 of PTZ seizure induction, plastic processes come into play associating stimuli evoked responses with circuits involved in the ictogenic process. In spite of the enhanced preseizure evoked response discussed above, there was no indication that stimulation interfered with the onset or severity of the PTZ-induced seizures in any of the recorded groups, i.e., no evidence for a kindling effect [35].

There is quite an extensive amount of data suggesting that TH stimulation has anticonvulsant properties in different etiologies of epileptic conditions [36]. In fact, some authors have even suggested that the lesion caused by TH electrode placement might itself reduce the occurrence of seizures [37]. One possible explanation for these results is the diffuse connectivity, throughout multiple inner cortical pathways, that may compromise the abnormal coupling of several microseizure domains [14,38–42]. Altogether, data make a strong argument for using TH structures as active probing stimulation sites for seizure prediction although very few research has been done on the subject. Our results from Protocol-one add to these claims once TH electrode placement did interfere with PTZ seizure latency (Fig. 2B and 2F comparison — seizure threshold TH-PTZ-noDBS: 0.05 ± 0.01 g/kg and AMY-PTZ-noDBS 0.04 ± 0.004 g/kg) and also proved to be an effective seizure prediction probing signal (as seen in the Results section, Fig. 2).

In Protocol-two, evidence suggests that there could be long-term plastic changes caused by DBS during the initial stages of PTZ infusion — i.e., the B-ES group did not return to baseline stimulus response levels after day-1 (EST period at day-2 — Fig. 3C). These results are even more interesting considering that A-ES and ES groups did not present the same long-term effects. Accordingly, such data may reflect a diffuse association between stimuli and 1) several desynchronized networks (i.e., seizure-unrelated circuits) and also 2) seizure-related circuits, believed to be facilitated at the early stage of the ictogenic process [14,43,44]. In this case, the lack of association with a preferred circuit, thus, leading to signal dampening, would explain the lower energy levels of the evoked responses to stimuli with no PTZ (EST period). In contrast, if DBS is presented while a specific oscillatory circuit gains control of the network (electrographic epileptiform activity), i.e., ES group, it would be reasonable to assume that changes in evoked response levels would only occur when that specific circuit is once again driving the network activity (Figs. 3C and 4B). At last, the A-ES group does not change evoked response levels in the EST period perhaps for a different reason; no associative plastic process takes place during the postictal phase (Fig. 3C).

Although the aforementioned results are quite interesting, the central aim of the experimental design of Protocol-two was to investigate

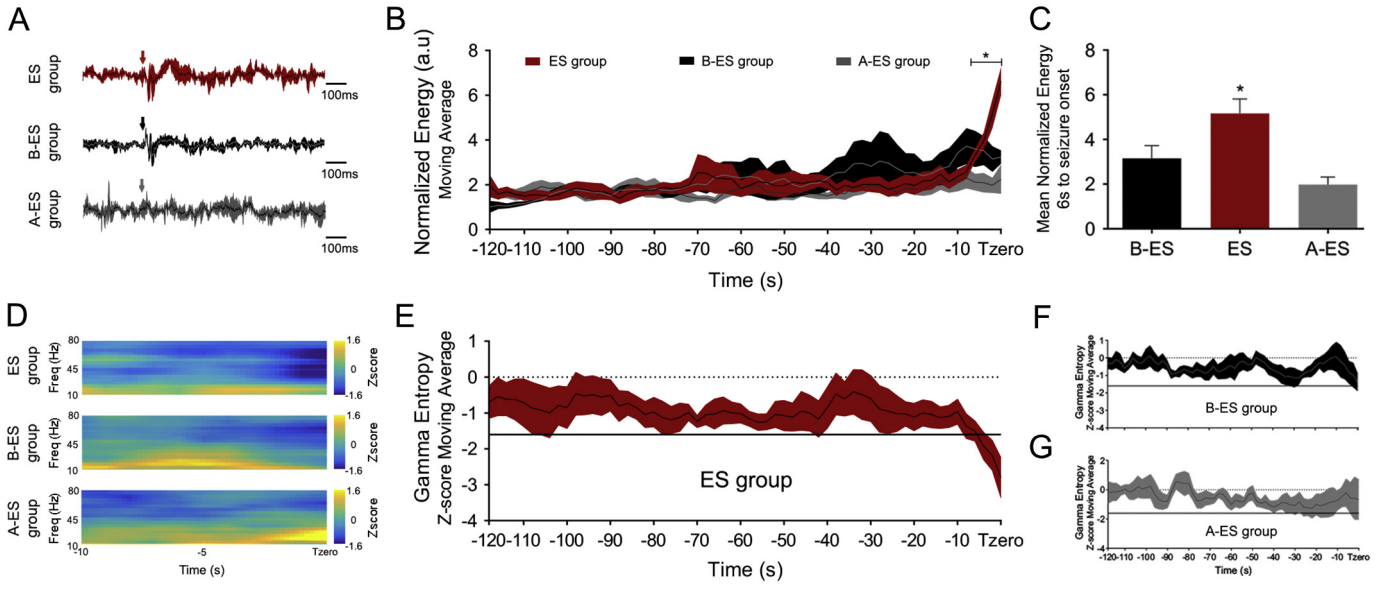

Fig. 4. Seizure prediction probing strategy applied at day-2. A: Representative LFP signal 6 s before seizure onset overlapped at a 2-second window periods. Arrows indicate the DBS timestamp. B: Normalized energy (moving average five elements) during the PTZ ictogenic progress (120 s prior seizure onset). * denotes $p < 0.05$ for group comparisons. C: Mean normalized energy value from the 6 s to epileptic polyspike discharges (Tzero). * denotes $p < 0.05$ for group comparisons. D: Z-score spectral entropy of the 10 s that precede seizure onset. E, F, and G: Z-score gamma (30–80 Hz) entropy during the PTZ ictogenic progress (120 s prior seizure onset). The black line represents the critical z-score value for a significance level of 95%.

a new strategy for active probing enhancement within the framework of the PTZ-induced ictogenic process. Nevertheless, the same rationale applies. The ES group was optimally designed to pair DBS to the dominant circuit (i.e., epileptogenic network); thus, as expected, it produced the most significant changes in the CRER. The B-ES group, as discussed, presented a partial association with the epileptogenic circuit but also with competing diffuse desynchronized circuits, therefore, presenting less evident, and much more variable, evoked responses. At last, the A-ES group, intentionally positioned outside the seizure circuitry activation window, had the least favorable results. In addition, the B-ES temporal evolution profile clearly differs from the other groups (Fig. 4B). In fact, the characteristic baseline fluctuation and high variance resemble the thalamic stimulation preseizure temporal evolution profile (Fig. 2D). Several researchers work with the hypotheses that one main epileptiform oscillator is gradually formed by hijacking multiple microdomain oscillators, abnormally strengthening their connectivity — in a winner-takes-it-all manner [14,40,45]. Accordingly, the fact that only the ES group showed a significant decrease in spectral entropy previous to seizure onset (Fig. 4D–E) favors that hypothesis. Additionally, it is relevant to highlight that oscillators are organizing around the gamma band, possibly associated with local circuitry activity [46], suggesting that the common attractor that is likely synchronizing the network has been highlighted by the pairing DBS. Previous results from our group have shown that even at the cost of increasing overall network excitability, DBS circuitry activation in a nonperiodic and/or desynchronized manner produces an anticonvulsant effect while periodic stimulation (four pulses per second — 4 Hz) increased circuitry coupling and, thus, anticipates seizure onset [41,47,48]. Although the anticonvulsant DBS is not within the scope or aim of this work, such a line of thought lays the theoretical foundation for explaining the great success active probing has brought to seizure prediction strategies [34], in contrast to the debated perspectives of using passive EEG analysis. In fact, results from human studies have already demonstrated the promising applicability of the probing DBS for cortical excitability evaluation [11] and forecasting the ictal activity [9,13]. Nevertheless, to the best of the author's knowledge, the present work is the first to associate the epileptogenic/ictogenic networks to desired circuits of interest per plastic modifications induced by seizure activity [49], which open a helm of possibilities for both diagnosis and treatment of epilepsy. The pairing DBS protocol could be used to enhance the evaluation of neural excitability and further applied to the neurological diagnosis of epilepsy. In addition, seizure control efforts could be associated with pairing-probe DBS in order to design a closed-loop system that would only intervene at the precise timing before the seizure, decreasing the collateral effects caused by chronic treatments. Despite the short preictal period detected (10 s), which might be extended by repeated DBS–seizure pairing, rapid antiepileptic actions as electrical stimulation [50] or optogenetics [51] could still be used in an effective feedback-control approach. Nevertheless, the immediate association with closed-loop solutions, although promising, must be complemented with further studies to address potential caveats. Just as an example, the “induced” coupling between ictal and nonictal circuitry may as well produce the undesired effect of spreading epileptiform activity to areas that, before entrainment, were deemed normal. However, it is equally possible that repeating the procedure of detecting and applying feedback stimuli intended to suppress seizure initiation (i.e., closed-loop solutions) could induce plastic changes that would stabilize the circuitry involved in ictogenesis, [52,53], potentially curing epilepsy. In fact, although as yet there is no conclusive evidence that the long-term use of responsive neurostimulation system (RNS) in clinical practice reverts the epileptic condition, there were suggestive indications of cognitive improvements in the patients [54] that could be associated with plastic changes induced by treatment. It should be added that with the present EEG probing enhancement technique, areas other than the possible foci can be targeted aiming to induce a more permanent long-lasting effect. However, such claims only highlight the need for further research on the subject.

5. Conclusion

Our work has shown that by pairing electrical stimulation and the epileptic discharges, the AMY probing strategy was able to elicit a detectable altered brain state prior to the onset of the polyspike activity. The implications of using a pairing approach to enhance the EEG probing effectiveness opens a helm of possibilities at epilepsy research, particularly in the seizure prediction efforts. Nevertheless, more studies must be performed to confirm and expand the promising results presented by this work.

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Conflicts of interest statement

The authors declare no conflict of interest.

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