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Anatomically dependent anticonvulsant properties of temporally-coded electrical stimulation

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ABSTRACT

In the PTZ animal model of epilepsy, electrical stimulation applied to the amygdaloid complex may result in either pro-convulsive or anticonvulsant effect, depending on the temporal pattern used (i.e. periodic-PS and non-periodic-NPS electrical stimulation). Our hypothesis is that the anatomical target is a determinant factor for the differential effect of temporally-coded patterns on seizure outcome. The threshold dose of PTZ to elicit forelimb clonus and generalized tonic–clonic seizure behavior was measured. The effect of amygdaloid complex PS on forelimb clonus threshold showed a pro-convulsive effect while NPS was anticonvulsant. NPS also significantly increased generalized tonic–clonic threshold; while PS, although at lower threshold levels, did not present statistical significance. Thalamus stimulation did not affect forelimb clonus threshold and showed similar anticonvulsant profiles for both PS and NPS on generalized tonic–clonic threshold. In summary, the anatomical target is a determinant factor on whether temporally-coded ES differentially modulates seizure outcome.

1. Introduction

Epilepsy is characterized by recurrent and spontaneous seizures caused by hyperexcitable and hypersynchronous underlying neural networks [1]. Despite the great advances of drug development over the last decades [2], pharmacological treatment is still unable to satisfactorily control seizures in about one third to one fourth of epilepsy patients [3–5]. Intracranial electrical stimulation (ES) is emerging as a new alterative approach for the treatment of pharmacoresistant epilepsy [6].

Classically, ES is believed to work either by suppressing or inhibiting epileptogenic structures, analogous to surgical ablation (high frequency stimulation), or by activating or stimulating neural networks that would modulate seizure-like activity (low frequency stimulation) [7–9]. The careful choice of parameters such as frequency, intensity and anatomical positioning of electrodes were believed to govern the ES usage as a seizure-suppressing procedure. Nevertheless, previous results from our laboratory, using the PTZ animal model of epilepsy, showed that a fixed 4-stimuli-per-second ES, in the amygdaloid complex (AMG), could either facilitate or interfere with the behavioral manifestation of the seizure, depending on how the stimulus was temporally coded [10,11]. Thus, the frequency parameter alone cannot explain the effect of ES on the PTZ seizure outcome, opening a new venue of possibilities in order to enhance ES efficiency as a therapeutic tool against epilepsy.

However, it has not yet been evaluated if other structures, besides the AMG, may respond to time-coded electric stimulation, differentially modulating seizure activity depending on the pattern of ES used. Our hypothesis is that not every structure will be able to decode time-patterns of ES. One such alternative target to temporally-coded ES is the thalamus (TAL), already tested throughout the literature (periodic high-frequency ES only) and showing positive results in seizure suppression [12]. Although the TAL has extensive connections with forebrain and brainstem regions [13], which may explain why thalamic ES is used in the treatment of pharmacoresistant epilepsy, its function and neural architecture differ greatly from that of the AMG. The objective of this work is to test whether time-coded ES in the thalamus has the same effect as that observed for the AMG, either facilitating (periodic stimulation) or interfering (non-periodic stimulation) with the behavioral manifestation of the seizure in the PTZ animal model of epilepsy.

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2. Methods

2.1. Electrical stimulation

We designed and built an electrical stimulator composed of a constant-current isolation unit driven by the output of an MP3 player (model NWZ-B152 2GB — Sony). Each output ES signal was designed using Adobe Audition 1.0 and transformed into a 44.1-kHz, 16-bit, mono waveform, MP3 format compatible with the D/A hardware output. Although constrained at a fixed total frequency of 4 stimuli per second, two patterns of temporally-coded stimuli were used: 1) constant inter-pulse intervals (IPIs) of 250 ms (periodic stimulus, PS); 2) randomized IPI (non-periodic stimulus, NPS) (Fig. 1). Each single stimulus consisted of a 350-μA square wave pulse of 100-μs duration. The temporal patterns used were chosen based on previous reports from our laboratory [10,11].

2.2. Animals

Male Wistar rats (n = 44; weighing 250–300 g), supplied by the CEBIO-ICB-UFMG vivarium, were housed under controlled environmental conditions (22 ± 1 °C), with a 12:12-h light–dark cycle and free access to food and water. All experiments were executed under Protocol License no. 150/06 approved by the university's Ethical Committee for Animal Experimentation (CETEA–UFMG). Efforts were made to avoid any unnecessary distress to the animals, and the lowest possible number of animals was used. The CETEA directives are in compliance with NIH guidelines for the care and use of animals in research.

2.3. Surgical procedures

Bipolar electrodes, 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 AMG (n = 24) and the TAL (n = 20). Animals were anesthetized by means of an i.p. injection of the mixture of ketamine (70 mg/kg — Pfizer, Karlsruhe, Germany) and xylazine (15 mg/kg — Bayer, Leverkusen, Germany) and positioned in a stereotaxic frame (Stoelting Co., Wood Dale, IL, USA). Coordinates for the anterior nucleus of the thalamus (AP = 1.3 mm, ML = 1.6 mm, referenced from the bregma suture, and 5.5 mm from dura mater) and amygdaloid complex (AP = 2.8 mm, ML = 5.0 and 7.2 from dura mater) were derived from the Paxinos and Watson's rat atlas [14]. The electrode was fixed to the bone with zinc cement and soldered to a telephone jack (Model RJ-11), which, in turn, was fixed onto the skull with dental acrylic. After surgery, animals received a prophylactic pentabiotic (2.5 mg/kg) treatment and were allowed to recover for 5 days before the experimental procedure. Groups were further subdivided according to the temporally-coded ES applied: no stimulus (AMG n = 12, TAL n = 9); PS (AMG n = 7, TAL n = 5) and NPS (AMG n = 5, TAL n = 6).

2.4. PTZ infusion

Before commencing the stimulation procedure, the caudal vein was cannulated for intravenous infusion of PTZ (10 mg/ml — Sigma) diluted in saline. The cannula was connected to an infusion pump set at the rate of 1 ml/min. Results were expressed as the PTZ threshold dose normalized by body weight (g/kg of animal) for forelimb clonus (FC) and generalized tonic–clonic (GTC) seizure onset. The choice of the former FC and GTC behavioral markers is based on the scoring scale proposed by Velisek et al. [15], in which FC would correspond to a fully developed minimal seizure (scale 3) while GTC would be a fully developed maximal seizure (scale 5). After stimulation, animals received an anesthetic overdose of urethane (140 mg/kg) before brain removal and histological procedures. Brains were sliced in order to confirm the electrode position. Animals with incorrect positioning of electrodes were not included in analysis.

Data are presented as means ± S.E.M. Statistical comparisons were made using one-way ANOVA and post-hoc Student–Newman–Keuls (SNK). The PTZ thresholds for both convulsive behavior markers, FC and GTC seizures, were compared according to the stimulus pattern (no-stimuli, PS and NPS). Values of $p<0.05$ were considered statistically significant.

3. Results

All animals displayed the typical convulsive behavior sequence of the PTZ model [15] 1) initial intensive grooming, sniffing, moving arrests; 2) followed by occasional isolated myoclonic jerks with ear and facial twitching; 3) clonus of the head muscles and forelimbs, and the presence of the righting reflex (the FC behavioral marker); 4) generalized clonus, without the tonic phase, and, finally; 5) the GTC that is usually preceded by a jump, followed by tonic falling and flexion or extension of forelimbs and hind limbs (maximum seizure).

Animals exposed to PS on AMG during PTZ infusion had significantly lower FC threshold ($FC_{PS} = 14.2 \pm 1.5$ mg/kg; $p<0.001$) when

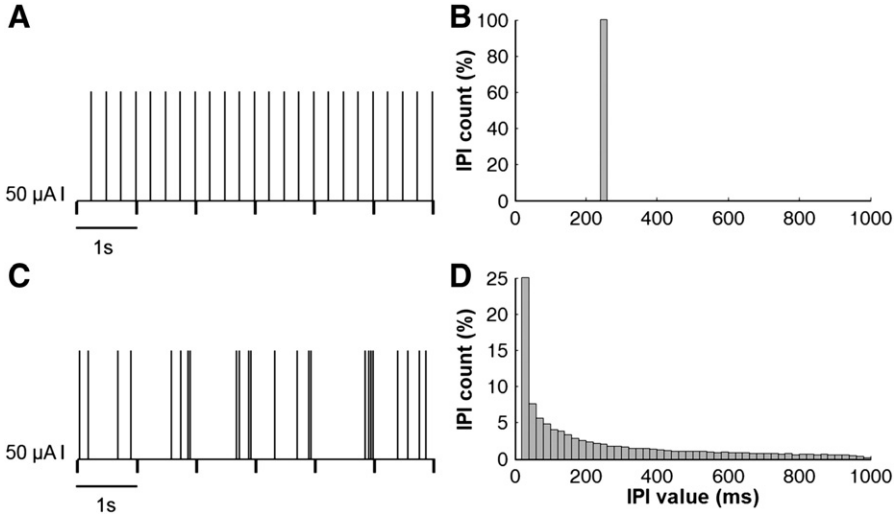

Fig. 1. Rats were stimulated with two different temporal patterns: (A) periodic (PS) and (C) non-periodic (NPS) electrical stimulation. The inter-pulse-intervals (IPI) for the PS and NPS are depicted respectively in histograms of occurrence B and D. Electrical stimulation, for both patterns, was characterized by a 350-μA, 100-μs duration and by four-stimuli-per-second pulse. Note that while PS has a fixed IPI (B) of 250 ms (4 Hz), NPS presents fairly randomized IPIs (see Cota et al. [10] for details).

compared to control ($FC_{control} = 22.8 \pm 1.2$ mg/kg) (Fig. 2A). In contrast, NPS displayed significantly higher threshold for both behavioral markers ($FC_{NPS} = 38.3 \pm 1.7$ mg/kg; $p<0.001$ and $GTC_{NPS} = 72.2 \pm 7.9$ mg/kg; $p<0.01$) (Figs. 2A and B) compared to controls ($FC_{control} = 22.8 \pm 1.2$ mg/kg and $GTC_{control} = 53.1 \pm 5.5$ mg/kg) (Figs. 2A and B). In addition, NPS and PS groups were significantly different for both FC ($p<0.001$) and GTC ($p<0.01$) [FC (Fig. 2A); one-way ANOVA: $F[2,21] = 50.21$, $p<0.0001$; GTC (Fig. 2B); one-way ANOVA: $F[2,21] = 3.78$, $p<0.0394$].

No significant effect was observed, for thalamic PS and NPS, on FC seizure threshold when compared to control. However, GTC threshold was significantly higher for both PS and NPS ($GTC_{PS} = 70.5 \pm 4.0$ mg/kg; $GTC_{NPS} = 70.3 \pm 3.9$ mg/kg) groups when compared to controls ($GTC_{control} = 49 \pm 2$ mg/kg) [FC (Fig. 3A); one-way ANOVA: $F[2,17] = 0.24$, $p = 0.78$; GTC (Fig. 3B); one-way ANOVA: $F[2,17] = 13.81$, $p = 0.0003$].

Although the mechanical lesion due to electrode insertion is easily visible in histology, there was no significant difference between AMG and TAL control groups (none of which was submitted to ES, but both were implanted with the same electrodes as the experimental groups). Thus, although this may be an issue for other studies, this particular work refrained from further discussion on the matter.

4. Discussion

The results confirm that distinct temporal patterns of ES, when applied to the AMG, differentially modulate seizure outcome in the PTZ continuous infusion model (i.e. PS had pro-convulsant properties while NPS was anticonvulsant). These results are in accordance with previous data from the literature [10], which suggest that PS would resonate with epileptogenic circuits, thus facilitating seizures outcome; while NPS would desynchronize circuits and interfere with neural recruitment necessary for the epileptic process. However, TAL ES did not significantly affect FC seizure threshold and had an anti-convulsant effect on GTC threshold for both temporally-coded ES patterns used (PS and NPS). In fact, the relevance of data presented here is significantly increased based upon the logical sequence of prior publications [10,11].

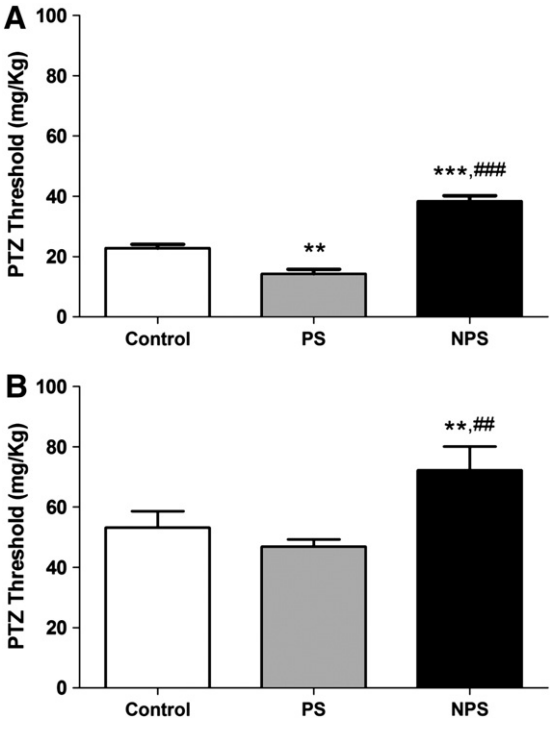

Fig. 2. PTZ threshold (normalized by body weight) for two convulsive behaviors: forelimb clonus (A) and generalized tonic–clonic seizures (B). Periodic stimulation (PS) and non-periodic stimulation (NPS) patterns were applied to the amygdaloid complex. Periodic stimulation (PS) decreased PTZ threshold for forelimb clonus (pro-convulsant effect). Non-periodic stimulation (NPS) increased PTZ threshold for both forelimb clonus and generalized tonic–clonic seizures when compared with all groups (anticonvulsant effect). $^{**}p<0.01$, $^{***}p<0.001$ periodic and non-periodic vs. control group, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ periodic vs. non-periodic in one-way ANOVA, post-hoc SNK. See the Results section for the numerical values of bars from this figure.

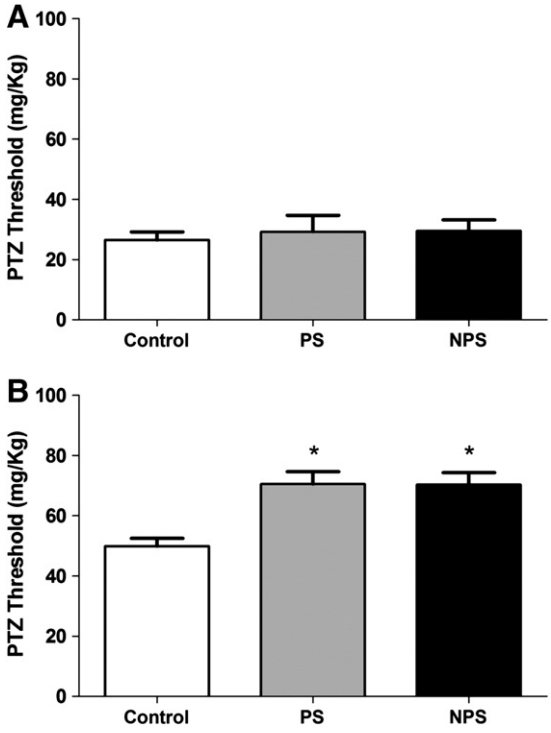

Fig. 3. PTZ threshold (normalized by body weight) for two convulsive behaviors: forelimb clonus (A) and generalized tonic–clonic seizures (B). Periodic stimulation (PS) and non-periodic stimulation (NPS) patterns were applied to the thalamus. No difference was observed between PS and NPS for forelimb clonus threshold. However, both the PS and NPS increased drug threshold for generalized tonic–clonic seizure when compared with control group (anticonvulsant effect). $^{*}p<0.05$, periodic and non-periodic vs. control group in one-way ANOVA, post-hoc SNK. See the Results section for the numerical values of bars from this figure.

Although PTZ creates a nonspecific condition of hyperexcitability, in part due to its GABAergic antagonist properties [16], evidence suggests that multiple neural circuits are gradually recruited into the ictogenic process as the drug is absorbed. In fact, low doses of PTZ (<40 mg/kg) typically evoke minimal seizures (i.e. myoclonic jerks, forelimb and head clonus [15]), which are classically correlated with limbic structures [17,18]; while higher doses of PTZ evoke maximal seizures (i.e. generalized tonic–clonic behavior), which are most likely correlated with brainstem activation [19]. The precedence of forebrain recruitment over brainstem substrates in the PTZ-induced seizures is suggestive of a higher threshold of the latter when compared to the former [19]. However, the view that these two substrates are completely independent seizure generators is not supported the literature [20,21]; in fact, the interactions between forebrain and brainstem seizure networks, under certain conditions, have an important overall modulation on seizure outcome. As an example, in an epileptic animal model of repetitive brainstem seizures, induced by high intensity sound stimulation [22], forebrain circuits are secondarily recruited after 13–17 audiogenic seizures. However, once recruited, forebrain circuits inhibit GTC seizures and generalized electrographic activity, maintaining only focal temporal lobe epileptiform discharges.

Our results show that ES has a clear modulatory effect in forebrain circuits only when applied to the AMG, based on FC onset data (Fig. 2). In addition, brainstem circuits seem to be affected by both AMG and TAL ES, however, possibly by two different mechanisms: a) in the case of AMG ES, forebrain synchronization/de-synchronization modulates brainstem circuits and b) the TAL ES directly interferes with brainstem neural recruitment without requiring forebrain.

The amygdaloid complex plays an important role in modulation and transfer of epileptiform activity in several animal models of temporal lobe epilepsy [23–26]. The AMG has monosynaptic afferents from and efferents to the parahippocampal areas (e.g., entorhinal cortex and subiculum) [27], providing the anatomical substrate for transfer and modulation of epileptiform activity. These connections provide an explanation for the pattern dependent effect of AMG-ES in FC threshold and increased GTC threshold [11]. One possible explanation as to why AMG PS did not significantly alter seizure threshold for GTC, since it did have a pro-convulsant effect in FC, is that forebrain modulation in epileptogenic brain-stem circuits would be primarily inhibitory [19]. Also, it is important to highlight that PS and NPS may have similar consequences in the underlying circuitry excitability but rather different effects on neural synchronization [11].

The thalamus provides the major inputs to cortex and primarily, but definitely not exclusively, working as a relay nucleus, which integrates and passes information from primary sensory modalities, basal ganglia, cerebellum, and the limbic system [28]. Due to its extensive connections, it is not surprising that the thalamus plays an important role in the abnormal synchronization between cortical and subcortical structures in tonic–clonic seizures [29]. Therefore, TAL ES, in contrast to AMG ES, seems to directly inhibit epileptogenic brainstem seizures, independently on the degree of synchronization imposed by PS/NPS, which is comprehensible, considering that this structure may relay information to the same output independently of the ES pattern used.

In summary, our results suggest that the amygdaloid complex is capable of decoding temporal arrangements of stimuli paradigm, which, in this case, may play an important role in desynchronizing epileptic seizures, especially forebrain seizure-like activity.

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