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Nonperiodic stimulation for the treatment of refractory epilepsy: Applications, mechanisms, and novel insights

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ABSTRACT

Electrical stimulation of the central nervous system is a promising alternative for the treatment of pharmacoresistant epilepsy. Successful clinical and experimental stimulation is most usually carried out as continuous trains of current or voltage pulses fired at rates of 100 Hz or above, since lower frequencies yield controversial results. On the other hand, stimulation frequency should be as low as possible, in order to maximize implant safety and battery efficiency. Moreover, the development of stimulation approaches has been largely empirical in general, while they should be engineered with the neurobiology of epilepsy in mind if a more robust, efficient, efficacious, and safe application is intended. In an attempt to reconcile evidence of therapeutic effect with the understanding of the underpinnings of epilepsy, our group has developed a nonstandard form of low-frequency stimulation with randomized interpulse intervals termed nonperiodic stimulation (NPS). The rationale was that an irregular temporal pattern would impair neural hypersynchronization, which is a hallmark of epilepsy. In this review, we start by briefly revisiting the literature on the molecular, cellular, and network level mechanisms of epileptic phenomena in order to highlight this often-overlooked emergent property of cardinal importance in the pathophysiology of the disease. We then review our own studies on the efficacy of NPS against acute and chronic experimental seizures and also on the anatomical and physiological mechanism of the method, paying special attention to the hypothesis that the lack of temporal regularity induces desynchronization. We also put forward a novel insight regarding the temporal structure of NPS that may better encompass the set of findings published by the group: the fact that intervals between stimulation pulses have a distribution that follows a power law and thus may induce natural-like activity that would compete with epileptiform discharge for the recruitment of networks. We end our discussion by mentioning ongoing research and future projects of our lab.

1. Introduction

Despite considerable development of both pharmacological and surgical treatments, a large portion of patients with epilepsy cannot obtain full control of their seizures [1,2]. In fact, optimistic statistics suggest there are at least fifteen million individuals in the whole world experiencing the neurobiological, economic, and social burden of pharmacoresistant epilepsy [3,4]. Alternative therapeutic approaches for refractory epilepsy are in obvious demand, and electrical stimulation (ES) is a promising choice [5,6].

Therapeutic electrical stimulation may be applied to targets of both the peripheral nervous systems — such as the vagus nerve [7] or the trigeminal nerve [8] — and the central nervous system (CNS). In the CNS, common therapeutic targets are the cortex surface, the cerebellum, anterior nucleus of the thalamus, subthalamic nuclei, and the epileptogenic focus itself, including the amygdaloid complex and the hippocampus in temporal lobe epilepsy [9]. Electrical stimulation is most commonly delivered as continuous or intermittent firing of square pulses of voltage or current in a certain fixed rate or frequency [10]. Although parameters vary a lot among different studies in humans and animal models, common values are units of volts or tens to hundreds of microamperes in amplitude and tens to hundreds of microseconds of pulse duration. Moreover, high frequency stimulation (HFS), with firing rates above 130 Hz, are broadly accepted as anticonvulsant and thus used in medical practice, while low-frequency stimulation (LFS; firing rates <20 Hz) is seen as proconvulsant or of controversial results [11,12].

The prevalence of HFS in clinical approaches is not without concern, since LFS would certainly be preferable because of the many advantages of a lower transfer of energy to the tissue. First, an implantable

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stimulator running LFS would need a smaller battery that would last longer, implying a less invasive surgery and fewer interventions for replacement. Second, a lower count of pulses per unit of time directly translates to decreased charge density around electrode contacts. This means improved therapeutic safety, since it reduces risks of nonsurgical-related lesions, such as those resulting from heat and electroporation. Also, LFS may be less prone to tissue habituation that results in loss of therapeutic effect in the long run. Finally, HFS, particularly when applied to areas of the mesial temporal lobe, is the protocol of choice for inducing long-term potentiation and kindling in experimental epilepsy, both related to increases in neural excitability, while LFS applied to the same areas has been described as an approach to induce long-term depression that may underlie antiepileptogenic effects seen in some studies. But again, seizure-suppressing effects of LFS are controversial at best [13–17].

It is thus of paramount importance for the advancement of neurostimulation therapy regarding its efficacy, efficiency, safety, robustness, and scope of application, to be able to reconcile the evidences of beneficial effects with the mechanisms of epilepsy, ictogenesis, and seizure abatement [18]. Our group has been pursuing this goal for more than a decade now by investigating an often-overlooked aspect of epilepsy beyond ubiquitous hyperexcitability: neural hypersynchronization. In 2009, we published, in a special edition of this prestigious journal for the NEWroscience 2008 International Symposium, evidence that a nonstandard form of LFS specially tailored to break reverberation in neural networks and thus to suppress excessive synchronization has a robust anticonvulsant effect in rats submitted to a model of chemically induced acute seizures [19].

Much work has been done since then. Here, we review the progress made in the science and technology behind this experimental therapeutic approach, termed NPS (nonperiodic stimulation), by developing the method and maximizing its effect, expanding its applications, and investigating its mechanisms. A review on the formal concept of neural synchronization and the techniques to measure it is given, before proceeding to the description of the findings of the group and the current understanding of the underpinnings behind the therapeutic effect of NPS. We conclude by describing ongoing investigation and perspectives of the work.

2. Some nuts and bolts of epilepsy: molecules, cells, and networks

A myriad of neurobiological factors from the molecular, to cellular, and to network levels of brain organization underlie the cardinal feature of hyperexcitability of epileptic neural tissue [20–24]. Among these, enhanced NMDA-mediated excitation [25] and decreased GABA-mediated inhibition [26], probably resulting from status epilepticus (SE)-induced trafficking of receptors through the membrane of postsynaptic neurons [27,28], are of key importance to the unbalance between excitation and inhibition, in favor of the former. Deficient potassium buffering, ion transporter dysfunction [29], impaired glial function [30,31], cell swelling [32], dendritic anatomical abnormalities [33], dendritic channelopathy [34], and many others are additional contributing factors in the plethora of neurobiological mechanisms underlying epilepsy. These pathological changes add up to create a permanent state of hyperexcitable neural tissue prone to develop episodes of aberrant intense neural activity: seizures. The dynamic transition from controlled hyperexcitability to a seizure state and back is not yet fully understood, but seizure onset and termination has been investigated and modeled as the movement of a particle in a surface to and from distinct wells of attraction representing the functional and the dysfunctional states, the separation of which is much decreased in epilepsy [35–39]. For instance, Suffczynski and colleagues have provided valuable insight on epileptic phenomena while investigating in silico the transition between dynamics underlying healthy spindle oscillations and dysfunctional spike and wave discharges by a bistable neuronal network model [40].

After the threshold is crossed, aberrant epileptiform activity spreads through both physiological pathways [22,41–43] and aberrant connections [44,45]. These neural networks are, thus, responsible for the propagation and sustaining of seizures [46–48] giving rise to distinct electrophysiological and behavioral expressions according to the substrates involved and the temporal coordination of their mutual activation [49–51].

It becomes clear at this point that both the disease and its main expression are complex phenomena encompassing multiple neurobiological factors in different levels of brain organization that demand alternative investigative approaches that are up to the task [52–54]. In this sense, it is important to understand that complex systems give rise to emergent properties that may contribute to the understanding of complex phenomena. One such feature is synchronism of brain activity. In fact, this property has been described to be inherent to neural networks in both homeostasis and dysfunction [55–57], including epilepsy [58,59].

By this token, epilepsy can be understood as a dysfunction of hypersynchronism (in most part at least). Although such understanding has been put forward since seminal work of Penfield & Jasper [60] and others, it has lacked a solid definition. Synchronization can thus be defined as the mutual influence of two dynamical systems entrained in oscillatory activity [61–63]. This means that if two oscillators are synchronized, at least a pair of any of their descriptors (amplitude, phase, frequency, etc.), one of each dynamic, will show a mathematical interrelationship of the form $y = f(x)$ [59].

Synchronization in epilepsy has been observed in multiple forms in different brain signals, such as noninvasive imaging, magnetoencephalography, and most predominantly — given its high temporal resolution — electroencephalography (EEG). A first evidence of neural synchronization is the occurrence of epileptiform spikes, which by itself is an indicator of simultaneous firing of neuronal populations making superficial synaptic contacts [64]. Furthermore, synchronization can be inferred simply by the spread and temporal locking of spikes among substrates of ictogenic circuitry [44,51,65,66] or by several approaches of electrographic signal processing, such as correlation measurements, partial directed coherence [67], phase-locking [68–70], phase lag [71], phase synchronization [72], cross-frequency coupling (CFC) [73–75], Granger causality [76], mutual information [77], and others [57,78].

Of particular interest, ES of the brain has been related to neural desynchronization [79,80]. On the other hand, the relationship between epileptic phenomena and its suppression by ES with neural synchronization and desynchronization, respectively, is not free of controversy, since it seems that increases and decreases in the coupling of oscillation depend on different factors, such as frequency of oscillation, instant in the course of ictogenesis, and spatial scale — short versus long range [59,81].

With the specific goal of decreasing synchronization in the low frequency range seen in animal models of generalized seizures [50], we designed NPS and tested it with different parameters and in distinct animal models of seizures and epilepsy, while, at the same time, investigating its therapeutic mechanism in face of the desynchronization hypothesis. The following sections of this text describe published findings and ongoing research of our group on the topic.

At this point, it is important to mention that two forms of NPS were developed: NPS-IH and NPS-LH (reasons for acronyms will be clearer later). Yet, only NPS-IH was shown to be effective and then it was renamed to just NPS for simplicity reasons. Thus, usage of acronym NPS here and in other publication of the group always implies the IH variation, while the LH variation is described in extent: NPS-LH.

3. NPS: temporal pattern generation and parameters

A desynchronizing and antireverberation stimulation pattern should have no regular temporal structure. Thus, all intervals between any two firing pulses (IPI: interpulse interval) of NPS were randomized. To keep it low energy, the stimulation pattern contained only four pulses per

second in average. This frequency was based on common firing rates of epileptiform spikes seen in animal models of acute seizures. To be able to perform randomization on real time, the algorithm consisted of the following steps: 1) randomize, from a uniform distribution, a timestamp *t1* between 0 and 940 ms; 2) wait until *t1* and fire the first pulse; 3) randomize a second timestamp *t2* between *t1* and 960 ms; 4) wait until *t2* and fire the second pulse; 5) repeat steps 3 and 4 two more times, while always increasing 20 ms to the end of the randomization period; 6) always check for IPI smaller than 20 ms and adjust to this value if necessary. By following these steps, we guaranteed four pulses per second with a real-time randomization of IPI always greater than 20 ms, so no high frequency content was present; firing rate was, thus, always <50 Hz.

This pattern of stimulation was also called restrictively randomized (given that randomization period always depended on previous randomization results) or even NPS-IH. The abbreviation IH stems from inverse histogram, since IPI distribution of this pattern can be fit with great accuracy ($R^2 = 0.9781$; [19]) by an inverse function of the form:

$$f(x) = \frac{k}{x}$$

where $f(x)$ is the probability of an IPI of size x and k is a multiplicative constant dependent only on the number of classification bins and the number of stimulus pulses (Fig. 1B, panels g and h). This characteristic has been proven to be of great importance (see Section 5.3). Considering that the algorithm for the generation of the temporal pattern does not allow frequencies over 50 Hz (or IPI < 20 ms), and that there are always four pulses per each one-second time window, the domain of IPI values is:

$$20\ ms \le x \le 1860\ ms$$

Pulses were always square waves of 100 μs total duration, with current amplitudes varying from 100 to 600 μA, according to animals' susceptibility (assessed prior to the experiments). This set of parameters and the low frequency nature of NPS reduced charge per phase in a 1-second period to as low as 0.1 μC, among the lowest levels found in literature and well below the safety limits [12].

Stimulation was applied to the amygdala (AMY) since 1) it is a major player in seizure generation and spread [23]; 2) it processes incoming information from polymodal areas through the entorhinal cortex, working as a gatekeeper to mesial temporal lobe circuitry; 3) it is extensively connected to structures in the hindbrain, midbrain, and forebrain [82]; and 4) there are advantages in using the epileptogenic focus for stimulation, including better neurosurgical safety and others. Stimulation target varied only when specifically investigating anatomic aspects of NPS. In the same vein, some of its parameters varied in some studies to serve the purpose of answering specific questions of each phase of the investigation (see next section).

4. NPS has anticonvulsant properties in acute and chronic seizures

The first steps were to test NPS against experimental seizures. A common animal model for the screening of new drugs or novel therapeutics is the controlled intravenous infusion of pentylenetetrazole (PTZ) [83]. It is a nonspecific $GABA_A$ receptor antagonist which, when gradually administered, induces a condition of neural hyperexcitability that starts in more susceptible areas, such as the limbic system, and then progresses to the whole brain [49,84]. This ensures gradual recruitment of structures into aberrant epileptiform activity, resulting in animals displaying a stereotypical sequence of convulsive behavior that begins with facial automatisms, progresses to myoclonic jerks and forelimb clonus, and ends with generalized tonic–clonic seizures (GTCS). Since PTZ infusion rate is controlled and constant, the time to evoke each behavior corresponds to the amount of drug necessary to trigger seizures (minimal or maximal) — also called PTZ threshold — and variations of this parameter indicate an anticonvulsant or a convulsant effect of a candidate treatment.

In a first study of our group, NPS was applied to the right basolateral amygdala of male rats submitted to PTZ-induced acute seizures ([19]; Fig. 1A, but with no LFP recordings). Besides NPS-IH stimulated animals, four additional experimental groups were carried out: 1) control with no stimulation; 2) low-frequency (4 Hz) periodic stimulation (PS; Fig. 1B, panels a and b); 3) burst stimulation consisting of four pulses with a 20 ms IPI (Fig. 1B, panels c and d); and 4) stimulation with a different algorithm for randomization (also four pulses per second), called freely randomized IPI or, yet, NPS-LH, because of the fact that the IPI histogram can be fit by a linear equation of the form $f(x) = A - kx$ (Fig. 1B, panels e and f).

Results showed that NPS (particularly and only NPS-IH) has a robust anticonvulsant effect on both forelimb clonus and GCTS, since PTZ threshold was significantly increased for both behaviors (almost double for GTCS) in animals submitted to such stimulation pattern, while other stimuli displayed no effect or were even proconvulsant (PS; Fig. 1C). Considering that every other important stimulation parameter (mean pulse amplitude and duration, number of pulses per second, stimulation site, mean animal weight, etc.) was the very same across all groups, we concluded that the temporal pattern of stimulation is a key determinant factor for the therapeutic effect. Curiously, only one form of randomization (NPS-IH) displayed an anticonvulsant property. Although this still remains to be fully understood, plausible neurobiological factors originating from independent sources of different neuroscience fields have come to light recently with great explanatory potential. We will come back to this later in the text.

Next, we sought to test NPS in a scenario that better mimics the human condition. For this, we submitted animals to the experimental model of temporal lobe epilepsy, represented by the late phase after pilocarpine-induced status-epilepticus (SE). Pilocarpine (PILO) is a cholinergic agonist that, when administered in a massive bolus intraperitoneal injection, induces SE which, in turn, if sustained for about 90 min or more, will cause neuronal damage and consequently maladaptive cellular and network restructuring. The final result in the long run is a dysfunctional neural tissue sustaining a permanent condition of hyperexcitability. Animals, thus, display spontaneous and recurrent seizures with concomitant recruitment of mesiotemporal regions and the limbic system [85–89].

In this study, our group applied NPS for 6 h (10 a.m. to 4 p.m.) during four consecutive days right after four initial days of observation (no stimulation), all of them in the late phase of the PILO model (45 days after SE), while assessing number, duration, and severity (according to Racine's scale) of seizures [90]. Compared with the control period with no stimulation, NPS was able to significantly reduce the number and duration of seizures, with a strong tendency to decrease severity (Fig. 1D). Periodic stimulation was also applied to a different group of animals in the same way NPS was: while results were not significant, a trend to worsen seizures was observed. We considered these to be important results, since NPS was effective even when applied to dysfunctional tissue. Not only does this support the possibility of a translational study and application of NPS to human patients, but it also suggests that its therapeutic mechanism is not lost in dysfunctional hyperexcitable neural tissue.

Based on the rationale that NPS attains its therapeutic effect by means of neural desynchronization, we then tested the effect of varying key stimulation parameters in the suppression of acute PTZ-induced seizure [91]. We analyzed the number of pulse phases (mono and biphasic), stimulation side (right and left), number of sides (uni- and bilateral), and synchronicity between hemispheres (synchronous and asynchronous; Fig. 1E). All these variations were known to have differential effects regarding efficacy and site of neuronal activation, quality of charge distribution, desynchronization power, etc. Data showed that anticonvulsant power increases gradually if biphasic, bilateral,

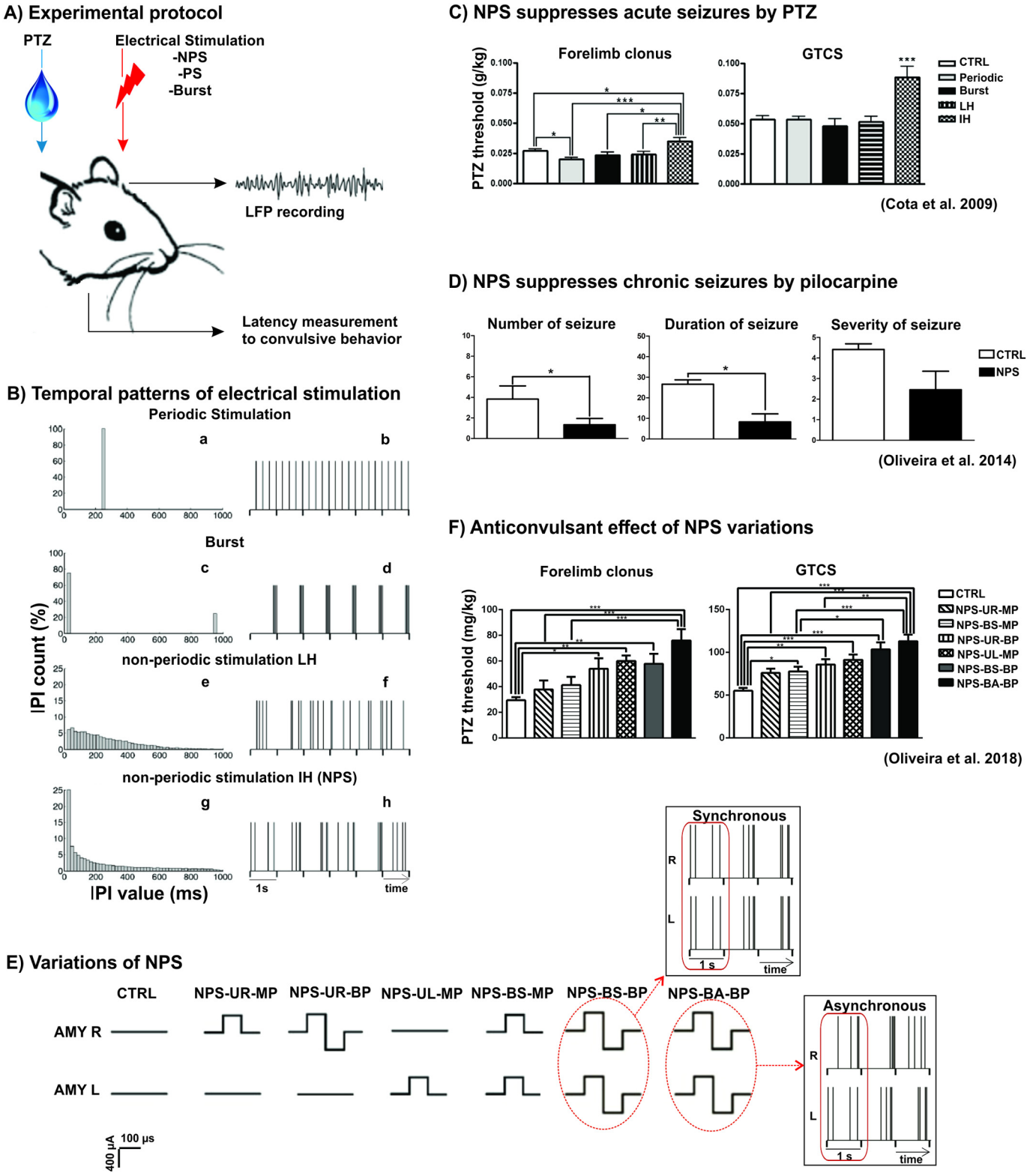

Fig. 1. Compilation of findings on the anticonvulsant effects of NPS. A) Wistar rats were submitted to PTZ-induced acute seizures while being treated with different temporal patterns of stimulation. Latency to convulsive behavior and electrophysiological recordings were performed to assess anticonvulsant effect and functional correlates. Method for induction of spontaneous and recurrent seizure by PILO is not shown. B) The different temporal patterns of stimulation used in this investigation, with IPI histograms at left and temporal representations on the right. C) Results for PTZ-threshold to evoke forelimb clonus and GTCS. D) Results for number, duration, and severity of seizures in PILO animals treated with NPS. D) Distinct variations of NPS. E) PTZ-threshold results for each variation.
(Adapted with permission from Cota et al. [19] and Oliveira et al. [90,91].)

and asynchronous features are added to the stimulation pattern, resulting in almost a threefold increase of PTZ-threshold to evoke GTCS when compared with controls (Fig. 1F). If these additions are modeled as increases in spatial and temporal complexities of the pattern of stimulation, measured by, for instance, spectral entropy or Shannon entropy, a strong correlation between such measurements and the

anticonvulsant power of different variations of NPS can be seen ([92] — this issue).

Finally, we also studied if NPS was capable of impairing the progression of experimental epileptogenesis (transition of a healthy brain into a hyperexcitable seizure-prone one). This is plausible, since the development of the epileptic condition in a previously healthy brain may be interpreted alternatively as neural plasticity underlying an aberrant form of memory formation [93,94]. Memory formation is related to synaptic weights, the modulation of which depends on the coincidence between firing of interconnected neurons. In turn, such coincidence has been shown in silico to depend on the temporal structure of external stimuli [95]. For this investigation, animals underwent fast electrical kindling of the amygdala intertwined (or not) with NPS. Preliminary behavioral and electrographic results showed that NPS decreases the severity of behavior and duration of seizures after kindling, which suggest that NPS has also an antiepileptogenic effect (unpublished results).

These lines of evidence, taken together, show that NPS has a robust anticonvulsant effect against both acute and chronic seizures, while suggesting that it also has antiepileptogenic effects. Results of the pilocarpine model show that the mechanisms of NPS are preserved in dysfunctional neural tissue, thus strengthening the possibility of translational research. Finally, the method displays its greatest anticonvulsant power when applied biphasically, bilaterally, and asynchronously.

5. Therapeutic mechanisms of NPS

As stated before, it has always been a main goal of this scientific endeavor not only to develop an efficacious ES method, but also to engineer it with the neurobiology of epilepsy in mind, particularly regarding the aspect of hypersynchronism among substrates of ictogenic circuitry [96]. Distinct behavioral outcomes originating from changing regular (PS) to irregular firing of pulses (NPS) and the fact that NPS efficacy is increased by varying its parameters from mono- to biphasic, uni- to bilateral, and synchronous to asynchronous (interhemispheric synchronicity) may be seen as indirect evidences that, in fact, the approach attains it therapeutic effect through neural desynchronization. Yet, a more direct mechanistic investigation of these issues was of paramount importance.

5.1. The importance of anatomy

A first attempt to understand the mechanism underlying NPS therapeutic effect and to test the desynchronization hypothesis was to assess anatomic aspects regarding both 1) neural recruitment after application of stimuli to the AMY and 2) conversely, behavioral outcome of varying the substrate of application.

In the first case, functional magnetic resonance imaging (fMRI) was performed in rats submitted to application of NPS or PS to AMY with or without a controlled infusion of PTZ, using a 7 T horizontal small-bore scanner, with fast spin-echo multislice sequence for structural images and single-shot gradient-echo echo-planar sequence for functional images, both T_2-weighted [97]. Special carbon fiber stimulation electrodes were developed to avoid imaging artifacts. As expected, PTZ induced generalized activation in both stimulation protocols. On the other hand, while PS reinforced ipsilateral activation (in regard to site of stimulus application), NPS reduced it. Moreover, in PTZ-free animals, PS induced activation of the mesiotemporal region (e.g., thalamus and hippocampus) in the contralateral axis, and NPS recruited frontal areas, especially the nucleus accumbens. This last result has been of particular interest, since the nucleus accumbens has been enrolled in epileptic phenomena [98,99] and also has served as a target for electrostimulation experimental therapies [100,101].

To test the importance of the anatomical target of NPS, the same protocol of Cota and colleagues was repeated [19], but stimuli were applied also to the anterior nucleus of the thalamus (ANT) [102]. The choice of ANT in this study is based on extensive previous literature showing the efficacy of ES applied to this substrate [15,103–107]. Results for the amygdala were the same as those obtained in a previous study of the group. On the other hand, NPS applied to ANT showed an anticonvulsant effect only regarding GTCS, but not forelimb clonus. This difference may be explained by the involvement of both structures in each type of seizure: while AMY participates in forebrain ictogenic circuits related to minimal (forelimb clonus) and modulates those of maximal (GTCS) seizures, ANT is related only to midbrain and hindbrain generalized seizures [108–111]. Corroborating this understanding, while PS showed a convulsant effect towards forelimb clonus when applied to AMY, it showed no such effect when ANT was the target. Conversely, PS at AMY had no effect at GCTS but it was anticonvulsant when applied to ANT.

These results highlight the importance of anatomy in the mechanism of ES in general and NPS in particular. Not only do different stimulus patterns applied to the AMY induce differential neural recruitment, but also the same pattern applied to different anatomical substrates yields distinct behavioral outcomes. This corroborates the notion that NPS has an effect on the network level of brain organization and that the amygdala, but not the thalamus, is capable of producing different responses according to the temporal pattern of the presented stimulus. This fact has been successfully used in a study relating active-avoidance learning conditioned by temporally coded electrical stimuli [112].

5.2. Multi-level desynchronization

In order to test the desynchronization hypothesis more specifically, electrophysiological recordings had to be carried out simultaneously to electrical stimulation in animals submitted to experimental models of seizures and epilepsy. Different setups and signal processing strategies allow inferences regarding synchronization levels in the microdomain (local neuronal populations and microoscillators), medium range domain (neural networks), and macrodomain (coupling of networks).

The first study of our group with such goal sought to better understand the role of the nucleus accumbens in epileptic phenomena and in the seizure-suppression mechanisms of NPS. Particularly, given the intriguing findings of our previous work [97], we wanted to test whether the substrate might serve as an indirect pathway for desynchronization of the forebrain. In order to do this, we submitted rats to the PTZ model of acute seizures, with or without bilateral electrolytic lesion of the nucleus accumbens and treated or not with NPS [113]. Evaluation of convulsive behavior — including PTZ threshold assessment — and concomitant electrocorticographic (ECoG) recordings were carried out. Electrolytic lesion severely worsened PTZ-induced seizures, increasing duration of behavioral and electrographic manifestations, and decreasing PTZ threshold. Nonperiodic stimulation treatment in these animals had no overt effect whatsoever. In the same work, we assessed the occurrence and morphology of epileptiform spikes in order to investigate synchronization levels of neuronal populations [64]. After detecting spikes with a custom-build routine, total number, firing rate, amplitude, and slope of spikes were computed. It turned out that the nucleus accumbens lesion induced an increase in all these parameters, strongly suggesting an increase in the synchronization in the activity of neuronal populations responsible for spike generation in cortical levels. Nonperiodic stimulation in animals with intact brains (without nucleus accumbens lesion) showed mild to nonexistent decreases in the same parameters.

We have been also investigating the effects of NPS directly in the synchronization levels of neural networks. To do this, local field potentials (LFP) have been recorded from the cortex, hippocampus, and thalamus of rats submitted to acute PTZ-induced seizures and treated or not with NPS. To assess synchronization of a network, the level of epileptiform spike coincidence was measured by simply detecting the spikes

and computing their cooccurrence ratio (within a given temporal window) between a pair of recorded channels. Stimulated animals showed a consistently decreased level of ictal coincidence in several different sizes of time-windows ([114] — this issue).

The same dataset has been used to investigate CFC that may reveal synchronization levels between distinct circuits oscillating at different velocities. For this, the modulation index (MI) has been applied to measure how much the phase of a slow-frequency oscillation is coupled to the amplitude of a high frequency oscillation [115]. Untreated animals injected with PTZ showed a gradual increase of MI for many different pairs of frequencies throughout the controlled infusion period and before seizure onset. At the same time, MI of NPS-treated animals remained in baseline levels for much longer. Moreover, MI of treated animals during ictal periods (forelimb clonus and GTCS) was lower in comparison with that of nonstimulated rats ([116] — this issue). Curiously, NPS also induced an increase of MI between specific pairs of frequency bands in the higher portions of the spectrum.

Taken together, these results indicate that NPS does not exactly desynchronize circuits but, actually, sustains synchronization at baseline levels when the system is stressed with hypersynchronizing external stimulus (such as administration of PTZ). Moreover, this effect is observable in different levels of brain circuitry or domains: from local populations to the coupling of distinct networks. Curiously, this synchronization buffer effect is not ubiquitous in regard to the frequency spectrum, and NPS may actually induce synchronization in higher frequencies. Although a bit counterintuitive, this may actually be beneficial, since synchronization in higher frequency oscillations have been described also as a physiological rhythm in epilepsy [117]. Moreover, as mentioned before, seizures are episodes of hypersynchronous neuronal firing, but not in a ubiquitous fashion, and actually, desynchronization can also be observed during ictogenesis [59].

5.3. Not every desynchronization is the same: novel insights into the temporal structure of NPS

Nonperiodic stimulation was designed to lack rhythmicity and thus to be antiresonating or desynchronizing. This was the reason behind randomizing the intervals between stimulation pulses. In fact, NPS has been described in some of our studies as temporally unstructured electrical stimulation. The set of behavioral and electrographic evidences gathered in the studies of the group seems to corroborate the notion that the lack of rhythm in the stimulus may cause desynchronization of ictogenic neural networks leading to the suppression of seizures.

On the other hand, this theoretical framework was unable to predict some of the findings along the investigation, some of them being actually counterintuitive when seen from the straightforward "lack of temporal regularity induces desynchronization" perspective. For instance, when directly measured with electrophysiology, NPS seems to promote rather a resistance to synchronization, acting like a buffer for the feature, instead of a more intuitive desynchronization. In addition, NPS activates the nucleus accumbens (instead of desynchronizing or inhibiting it) in seizure-free animals and also induces CFC in higher frequency bands in animals submitted to PTZ-induced seizures. Finally, and maybe most importantly, current theory does not allow the understanding of why the two different IPI randomization protocols used to create NPS-IH and NPS-LH yield distinct therapeutic effects: the former is efficacious and the latter is neutral.

In our 2009 study, we speculated that, while both forms of NPS were sending random firing motifs to different microcircuits at distinct times, thus promoting desynchronization, only NPS-IH was doing it with the right frequency content, given that IPI distributions from the different ES patterns have distinct counts of shorter and longer intervals. It is clearer now that this is only partly true and that the efficacy of NPS in the IH form relies much more on the shape of its IPI distribution than on its frequency content. In fact, the shape of the IPI distribution of NPS of the IH type is closely related to the spatial and temporal organizations of the brain and also to features of many other complex systems: they all obey a power law.

Mathematically, a power law can be stated as a relationship between two variables y and f in the form of:

$$y = \frac{1}{f^{\beta}}$$

where β is a constant that can assume any nonnegative value. One should notice that this is exactly the function that fits the IPI distribution of NPS-IH, in the particular case of $\beta = 1$. The presence of a multiplicative factor k in the fit of NPS-IH has no real relevance, since the behavior of the power law is unchanged, or, as usually termed, it is scale-free. This fact can be stated also as:

$$P(kf) = k^{-\beta}P(f)$$

where $P(f)$ is the power law function of f. Also, it is important to notice that, in turn, NPS-LH cannot be fit by such relation.

Power laws can be used to describe a myriad of phenomena of complex systems in nature. For instance, it describes the distribution of assets in the banking system worldwide, distribution of crater sizes in the moon, distribution of the number of contacts in social networks, citations of scientific papers, and the frequency of words in a given idiom [118]. In the brain, not only does neural tissue generate firing patterns that can be modeled by power laws [119], but neural networks also seem to better respond and process activity in this natural form [120], implying that neural circuitry has evolved to better tune to the properties of the environment [121].

Responses of neural tissue to electrical stimulation varying in regularity have been directly tested. In a pioneering work, Mainen and Sejnowski observed spike trains in cortical neurons of rats elicited by injection of constant or variable currents, using a slice preparation and somatic whole-cell recordings in the current-clamp configuration. They found that neurons respond with much greater reliability when submitted to natural-like inputs distributed as filtered Gaussian noise (i.e., $1/f^{\beta}$) mimicking synaptic activity [122]. Inspired by this work, Gal and Marom used joint input–output statistics to assess spike trains of single isolated cortical neurons from newborn rats plated onto substrate-integrated multielectrode arrays (MEA) and submitted to different regimens of extracellular electrical stimulation, according to the regularity of their IPI. The stimulation pattern following a power law with unitary exponent ($\beta = 1$) induced spike activity with greater reliability — as measured by the correlation of firing rates of both input and output — in comparison with stimuli with constant IPI, with IPI distributed as white noise, or even those with IPI following a power law but with distinct values of β [123]. Finally, Scarsi and colleagues carried out a similar experiment using in vitro culture of neurons plated onto MEA and stimulated with different temporal regularity to demonstrate that neuronal networks also better follow stimuli with IPI distributed as a power law. Of particular interest, they also tested different values of β and also of mean firing rates. While the average frequency seems to play no relevant role, unitary exponent was the one that induced the greatest reliability between input and output activities [124].

Although such input–output relationship between stimulation and neural activity has been described in the single neuron level or, at most, in in vitro cultures of cells forming microcircuits, the idea may be extrapolated to in vivo, large-scale network settings. By this token, it is very plausible that NPS, as a natural-like, scale-free pattern of stimulation following a power law of unitary exponent, may be easily processed in and recognized by neural networks organized in multiple levels (from microdomain to large scale) in the brain. That would work even in dysfunctional hyperexcitable tissue, given only that it can perform its basic function, which is generally the case of patients with epilepsy. In turn, such property would promote a widespread and naturally desynchronized response that would compete with the pathological

hypersynchronous epileptiform activity. Stimulation effects would thus be expressed as a seizure suppression property behavior-wise. Moreover, such stimulation, instead of promoting increase or decrease of any specific oscillation, would rather reestablish healthy patterns of synchronism coordination that results from normal processing of information in the brain. Electrophysiologically, this would be expressed as a resistance to increase synchronism to aberrant levels and/or as a restoration of synchronization patterns related to homeostasis. All these aspects have been observed directly or indirectly in our previous and ongoing studies. Finally, this novel theoretical framework would help explain the activation of the nucleus accumbens in seizure-free animals submitted to NPS as seen in the fMRI study, given that the pattern may represent natural-like activity with variability or, in other terms, that seems like natural salience/novelty.

These are only speculations that still lack further evidence and a more definite proof. They are being put forward exactly to create debate. On the other hand, given that the theoretical framework of NPS as a power-law scale-free stimulation needs fewer elements to explain the set of evidences, Occam's Razor principle recommends a thoughtful consideration (Fig. 2). In fact, much of the ongoing research and also future work of our group is based upon this new reasoning.

6. Ongoing research and perspectives

An obvious step in NPS research is to apply it to patients with epilepsy. In order to do it in the safest way possible, we have been studying a myriad of neural functions in seizure-free rats submitted to such stimulation. It is plausible that such stimulation will not cause a significant side-effect since it emulates natural-like input. Using well-stablished behavioral methods, we found (unpublished data) that NPS applied to the amygdala has no overt significant impact on episodic memory, on ambulation, on social interaction, and also on baseline levels of anxiety. In the same study, another group of animals was submitted to long duration (up to 9 days, 6 h per day) LFP recordings from structures known to be involved in both epileptic phenomena and in the coordination of the sleep–wake cycle. When recordings are assessed as state spaces based on spectral content [125], data showed that sleep–wake cycle architecture of stimulated animals does not differ from that of naïve animals.

If this theoretical framework that establishes NPS as a scale-free natural-like input capable of restoring healthy levels of brain activity and synchronization is in fact right, it becomes very plausible that the approach may induce amelioration of symptoms in other neurological disorders. For instance, it is known that generalized anxiety/stress and related psychiatric disorders are the result of dysfunctional activity or, more specifically, hyperfunction of the amygdala [126]. Preliminary data (unpublished) from our lab have shown that restriction-induced chronically stressed rats treated with NPS performed better in the open-field and elevated plus maze tests.

Another interesting application field for NPS may be Parkinson's disease (PD). Being the result of neurodegeneration of dopaminergic cells in the substantia nigra, PD is characterized by motor function impairment such as postural instability, rigidity, tremors, and bradykinesia [127]. Electrophysiologically, PD is marked by increases of synchronization in the β range observed in areas such as the subthalamic nuclei, globus pallidus externus, and internus [128,129]; a rhythm with key roles in corticospinal integration; and motor function in general [130,131]. In our lab, we are currently reproducing well-stablished animal models of PD, such as

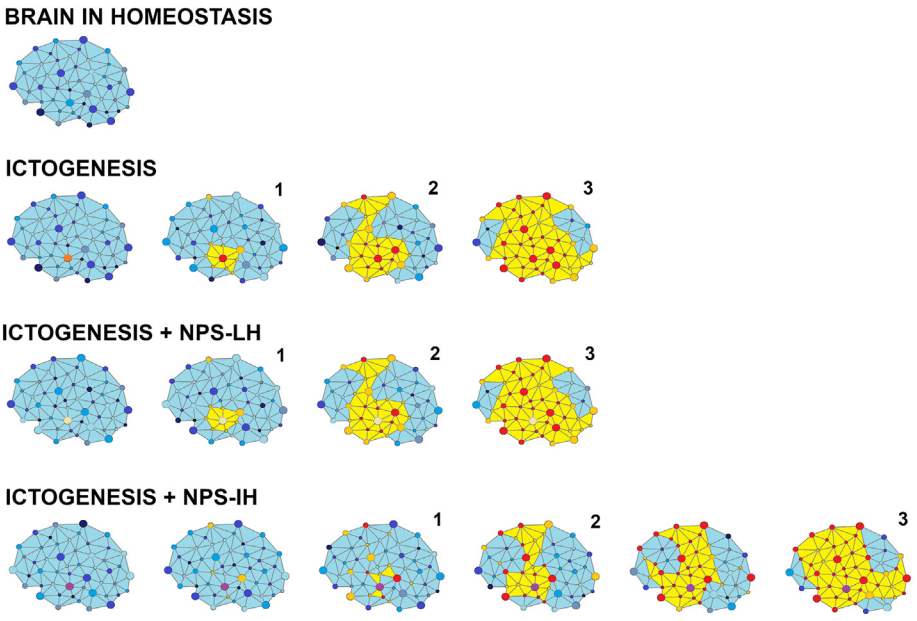

Fig. 2. Theoretical framework of NPS as scale-free temporal pattern that induces natural-like activity which, in turn, compete with epileptiform activity. Nodes (circles of different sizes) represent microoscillators, while edges are functional connections between a pair of nodes. Long-range connections are omitted for simplicity but considered in this rationale. Natural activity is depicted in shades of blue, while epileptiform activity in shades of red. A mix of them is represented in purple. Although the network in this figure is shaped like a human brain, we intend no correspondence with human neuroanatomy. **Row 1:** a brain in homeostasis displays widespread normal activity with spatial and temporal structure of a power law, together with normal levels of synchronization in its networks (triangles filled in blue). **Row 2:** ictogenesis induced by some endogenous (or exogenous) factor starts by a node (in orange) entraining in aberrant activity that will gradually spread to its neighbors. When a circuit is formed (step 1), epileptiform activity ensues (yellow fillings) and first mild motor manifestations are expressed (e.g., facial automatisms). Aberrant activity continues to spread, and when circuits encompassing major portions of the brain are entrained in aberrant activity (step 2), more severe convulsive behavior is manifested (e.g., forelimb clonus). Finally, when all major portions of the brain are recruited by epileptiform activity (step 3), generalized seizures occur. **Row 3:** when the epileptogenic focus is submitted to a pattern of stimulation that is not natural-like (e.g., NPS-LH), any effects gets territorially restricted to the point of application (beige dot) and ictogenesis progresses virtually in the same manner. **Row 4:** when NPS is applied to the epileptogenic focus of a brain during ictogenesis (purple circle), natural-like activity is spread through its connection and competes with epileptiform activity. Notice that, in each step, half of the nodes are recruited with epileptiform activity and the other half with natural like activity. This impairs the recruitment of networks into aberrant hypersynchronous oscillations, delaying occurrence of convulsion (steps 1 to 3). In every row, aberrant synchronization levels can be inferred by the number of circuit (triangle of nodes) filled with yellow. By this token, electrophysiological results indicate a resistance against synchronization increases.

application of electrolytic lesion or administration of 6-hidroxidopamine to one or both sides of the substantia nigra [132,133] to pursue such line of investigation. Since many of these alterations are also shared with the motor deficit outcome from brain ischemia followed by reperfusion, we may also follow a similar line of research using animal models induced by bilateral carotid artery occlusion [134].

Finally, it is of paramount importance to thoroughly test the hypothesis of NPS-induced natural-like activity suppressing epileptiform and other aberrant activity as a key therapeutic mechanism. We believe in silico investigation to be of central importance in this case. Particularly, in silico investigation would be highly beneficial in the rapid and ethical screening of variations of NPS (e.g., with different mean frequencies) or even other forms of IPI randomization that follow distributions related to the natural dynamics of the brain. In turn, not only would such screening advance the search for the optimal strategy of stimulation, but the comparison between distinct approaches may also provide insights on the mechanisms underlying the therapeutic effect of nonstandard stimulation and brain function in general. Alternatively, to close the loop and apply activity-dependent stimulation [135], responsive stimulation [136], or active probing for seizure detection or prediction [137] may also be of great value.

Declaration of competing interest

None.

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