





High and Low-Frequency Stimulation Effect on Epileptiform Activity in Brain Slices

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Abstract

Background: Neurostimulation is one of the new therapeutic approaches in patients with drug-resistant epilepsy, and despite its high efficiency, its mechanism of action is still unclear. On the one hand, electrical stimulation in the human brain is immoral; on the other hand, the creation of the epilepsy model in laboratory animals affects the entire brain network. As a result, one of the ways to achieve the neurostimulation mechanism is to use epileptiform activity models In vitro. In vitro models, by accessing the local network from the whole brain, we can understand the mechanisms of action of neurostimulation.

Methods: A literature search using scientific databases including PubMed, Google Scholar, and Scopus, using "Neurostimulation" and "epileptiform activity" combined with "high-frequency stimulation", "low-frequency stimulation", and "brain slices" as keywords were conducted, related concepts to the topic gathered and are used in this paper.

Results: Electrical stimulation causes neuronal depolarization and the release of GABAA, which inhibits neuronal firing. Also, electrical stimulation inhibits the nervous tissue downstream of the stimulation site by preventing the passage of nervous activity from the upstream to the downstream of the axon.

Conclusion: Neurostimulation techniques consisting of LFS and HFS have a potential role in treating epileptiform activity, with some studies having positive results. Further investigations with larger sample sizes and standardized outcome measures can be conducted to validate the results of previous studies.

Keywords: High-frequency stimulation, Low-frequency stimulation, Epileptiform activity, Brain Slice

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Introduction

Currently, medicinal and non-medicinal treatments are among the accepted treatments for this disease, but despite this (1), nearly 30% of epileptic patients are hurt by drug-resistant epilepsy. Neurostimulation of the epileptogenic zone is a new treatment approach for this disturbing disease. However, the mechanisms involved are still un-

clear (2-5). One way to study epilepsy and related therapeutic approaches are to induce epileptiform activities In vitro. In vitro preparations are extremely rapid, flexible, and available methods for long-standing problems in epilepsy research including ictogenesis and drug resistance. We have been able to gain molecular, cellular, and elec-

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↑What is "already known" in this topic:

Deep Brain Stimulation which typically involves Low-Frequency Stimulation and High-Frequency Stimulation is a new approach with many advantages for drug-resistant epileptic patients.

→What this article adds:

The effect of electrical high and low-frequency stimulation on neuronal excitability and seizure occurrence has been demonstrated in experimental models, but the precise mechanism has not been well-known. This investigation was proposed to consider the HFS and LFS inhibitory effects on neuronal excitability.

trophysiologic insights into brain function with detail that is not possible in vivo thanks to in vitro brain tissue preparations, which enable the suitable and reasonable research of brain networks (6). It has been possible to produce electrical activity in vitro that is similar to the electrographic activity found in individuals with epilepsy using preparations from both rodent and human postsurgical tissue.

Electrical stimulation, via targeting specific neural networks, is a modifiable and reversible treatment for patients with drug-resistant epilepsy. This strategy avoids undesirable consequences of resection surgeries that endanger patients' health (4, 7, 8). However, the exact stimulation protocols and the underlying mechanisms of deep brain stimulation, including high-frequency stimulation or low-frequency stimulation, are not still well known.

Synaptic activities and changes in the innate excitability of neurons have an important role in epilepsy occurrence. The In vitro brain slice is a perfect model for studying the effects of epileptogenesis and anti-epileptic interventions such as electrical stimulation of animal brain tissue (9, 10). It allows researchers to carry out elaborate studies on pathophysiology at a high level of molecular and electrophysiological resolution without disrupting the features of crucial networks of epilepsy. Therefore, In vitro brain slices are typically accepted as the best model for researching brain epileptic activity (11-13).

However, this approach has some drawbacks; the slicing process could injure tissue and isolates it from extrinsic bonds, which leads to lessening the local network available for investigation, and the occurrence of ictal seizure-like events is not spontaneously In vitro (14). Also, these preparations are usually used for many hours, so it's not beneficial for chronic epileptogenesis but just for acute provoked seizures studies (12, 15). Epileptiform activity was followed by increased neuronal firing and spike trains induced by epileptogenic agents in the brain slices (16-18). In this review, first, we will introduce and describe the different approaches to preparing epileptiform activity in brain slices. Then we will discuss the effects of low and high-frequency electrical stimulation on epileptiform activity.

General categories for epileptiform activity induction

For the first time, Li (1959) identified strychnine through intracellular signaling changes induced EEG interictal spikes in feline cortical neurons (19). before, he collaborated with McIlwain and showed cortical neurons could be sustained alive In vitro in a brain slice preparation (20). However, hippocampal slice preparations became well-founded In vitro tools to study cellular and pharmacological mechanisms complicated by epileptiform synchronization at the end of the 1970s (21).

The 1997 study by Khalilov et al. contributed significantly to In vitro brain research by demonstrating that the entire hippocampus (or even two interconnected hippocampi, including the septum) could be kept alive in a fully submerged chamber with separate compartments for drugs to be administered separately to each. There are different methods of epileptiform activity induction, and below we will discuss the various techniques for causing

them and the kind of epileptiform pattern In vitro.

GABA receptor antagonist

Epileptiform activity occurs following changes in the conductance of voltage-gated and ion channels. In the 1960s, epileptiform discharges were induced by using penicillin, and several investigators have shown, shortly after the early In vitro studies, that many compounds used for epileptiform induction were GABA_A receptor antagonists. Pharmacological manipulation changes that block GABA receptors produce epileptiform activity characterized by electrographic features like interictal activity, i.e., short-lasting (less than 3 s) synchronous field events that are intracellularly associated with large amplitude depolarizations and continuous action potential firing (22). The use of a GABA receptor blocker (23) such as pentylenetetrazole, picrotoxin, or other chemicals such as pilocarpine is less commonly used than the K⁺ or Mg²⁺ approaches. Pilocarpine, a non-selective muscarinic agonist, effectively exacerbates seizures in vivo but only exacerbates spontaneous epileptic discharge In vitro at much higher concentrations (24, 25).

High potassium concentration

Through a positive feedback mechanism, Traynelis et al. proposed that potassium levels gradually rise in CA1 during seizure periods. The next income-interictal burst precipitates a seizure when pyramidal cells are excitable to some threshold level. Seizures in high potassium may be a model of epileptic seizures or status epilepticus In vitro. It has been indicated that increased extracellular potassium concentration in hippocampal slices causes epileptic-like activity, which has several consequences being considered: first, the interactive input of CA3 to CA1 is necessary but not sufficient, as electrical convulsions could never occur in physiological potassium concentration normally (3.5 mmol). High K⁺ also induces a second interictal blast 30-60 seconds before the seizure by depolarizing pyramidal cells and glial cells in CA1 and extracellular space. These findings indicated a gradual accumulation of extracellular potassium concentration in the CA1 region before seizures (26, 27).

4-aminopyridine

Likely as the first investigators, Galvan et al. (1982) showed, bath applying the K⁺ channel blocker 4-aminopyridine (4AP) could induce spontaneous ictal-like discharges in slices of the guinea-pig olfactory cortex. In slow interictal discharges induced by 4AP, extracellular potassium levels increase transiently due to activating GABA_A receptors (28), and precisely because the K-Cl cotransporter isoform 2 (KCC2) co-transporter is activated, which increases extracellular potassium levels and chlorides (29). According to Avoli and de Curtis (30), increased extracellular K⁺ is associated with depolarization of neighboring neurons, ectopic spike formation, and a positive shift in the reversal potential of IPSPs mediated by GABA_A receptors, thereby weakening inhibition in these neurons. In the 4AP brain slice model, all of these mechanisms, initiated paradoxically by activation of

GABA_A receptors, are identified to raise neuronal excitability and stimulate ictogenesis. The potassium channel blocker 4-aminopyridine (4-AP) model of epilepsy has electrophysiological features, including the comparatively low frequency of 0.25 to 0.05 Hz occurring for 80 milliseconds. "Fast" pseudo-interictal events taking place at frequencies between 0.5 and 0.25 Hz for 500 mM initiate in CA3 and are primarily mediated by glutamate receptors; long-lasting ictal-like events in mature brains slices originate in the entorhinal cortex and are conveyed to the hippocampus (31-33).

Low Mg²⁺ concentration

Another approach to epileptiform activity induction is the elimination of Mg²⁺ from the artificial slice perfusion fluid because its blocking effect on the Mg²⁺-dependent block of NMDA (N-methyl-D-aspartate) receptors is removed, followed by ictal and interictal activity in the slice (34, 35). Following the removal of the Mg²⁺ characteristic of epileptic-like activity, the initial activity indicates the population at 50-300 milliseconds, and continued Mg²⁺ excretion leads to seizure-like events SLEs lasting several minutes. Intracellular recordings show large depolarizations (greater than 30 mV) with potential action onset firing, usually accompanied by rhythmic burst or discharge phase after discharge, leading to an asynchronous initiation pattern (35, 36). After 30 to 90 minutes of zero Mg²⁺ solution, there are no more ictal episodes; instead, there is a persistent rhythmic activity after discharge (35).

Low Ca²⁺ concentration

A non-synaptic epilepsy model in hippocampal slices has been established since the 1980s when low-calcium artificial cerebrospinal fluid solution prevented chemical synaptic transmission (37, 38). Individual bursts can last up to tens of seconds during this non-synaptic epileptiform activity. The activity is, therefore, along with the ictal epileptiform activity. A low Ca²⁺ induces epileptic activity through excessive excitation and hyper synchronization arising from non-synaptic mechanisms. A decrease in Ca²⁺ concentration and an increase in K⁺ concentration are associated with after-discharges in the hippocampus in vivo (39). Slow-wave and late-burst activity induced by low-calcium are morphologically similar to seizures caused by hippocampal epilepsy. The mechanism of epileptic action following calcium removal reduces surface charge screening, which may seem unusual because extracellular Ca²⁺ is essential for controlling vesicle fusion and neurotransmitter synaptic diffusion (40). It can be said that local field effects lead to ephaptic pairing and increased synchrony of neurons (41). Distinctive features of this model are epileptic-like activities that last for tens of seconds and are associated with neuronal depolarization and action potential firing that may be organized in rhythmic bursts or subsequent discharges. It is thought that this model could well express the ictal phase of seizures, in addition to showing extracellular Ca²⁺ drops to 100 μM during seizures in primates (42).

Electrical stimulation

Electrical stimulation is one of the most widely used seizure models in vivo. However it is less frequently employed in In vitro models. According to studies, this model has the following characteristics: (1) additional discharges just after the train. (2) The number of population increases that spontaneously explode. Explosions brought on by a single stimulus, and (3) Aside from that, all epileptic activity continued for up to 3.5 hours after the last train (43).

LFS effect on epileptiform activity

Furthermore, In vitro epileptiform activity models offer a unique opportunity to study focal seizure mechanisms in detail, especially when electrophysiological studies are involved. In vitro slice studies investigating the effects of interictal stimulation on seizures in epileptic areas were the first to examine the impact of low-frequency stimulation on epileptiform activity. Low-frequency stimulation (≤5 Hz, LFS) is a hopeful therapeutic strategy for epilepsy. In this regard, Bragdon et al. replaced seizure activity with interictal bursts in brain slices containing the entorhinal and hippocampal cortex using a zero mg²⁺ medium. They found that repeated stimulation mimicking interictal bursts (<2 Hz) suppressed seizure activity (44). Several studies have also investigated low-frequency stimulation in rat and mouse brain areas with interictal frequency mimicking strategies for mesial temporal lobe epilepsy MTLE in In vitro models. In the following and Table 1, we review and discuss their results.

Jerger & Schiff demonstrated that the initiation of tonic phase seizures in CA1 was suppressed when the CA3 (mossy fibers and Schaffer collaterals) was stimulated with frequencies of 1 or 1.3 Hz in the high potassium hippocampal slice. They demonstrated that in addition to periodic pacing, much more advanced techniques might be successfully used to regulate epileptic foci in experimental models. Furthermore, anti-control has reduced the frequency of such neuronal circuits by using control of chaos techniques. As a bonus, the power of chaos strategy uses the fewest stimuli necessary to achieve control, thus reducing the risk of epilepsy (45).

In another investigation, slices from adult mouse hippocampal-entorhinal cortex were exposed to Mg²⁺-free or 4-AP, which generated epileptiform activity. When low-frequency stimulation at (0.25-1.5 Hz) resembles the interictal activity of the CA3 area applied to the hippocampus output region in the entorhinal cortex suppresses the development of ictal discharges (46).

It has been shown by Khosaravani et al. that applying low-frequency stimulation (0.5 Hz, 20–the 50s) to the mossy fibers inhibits the conversion from interictal activity to seizure-like activity in dentate neuron axons. They hypothesized that during non-seizure epochs, brain activity could display complex dynamics (e.g., chaotic) but stabilize transiently in specific metastable periodic orbits within the chaotic attractor, representing interictal or ictal activity (47). Repeated stimulation of the basolateral nucleus of the amygdala at frequencies of 0.5 Hz and 1 Hz (which mimics CA3-driven interictal activity) reversibly

Table 1. LFS effect on epileptiform activity

Epileptiform activity type	Area	LFS criteria	Effect	Reference
Mg ²⁺ -free medium	hippocampus-entorhinal cortex	Interictal-rate	Suppression	(5)
K ⁺	CA3	1 and 1.3 Hz	Suppression	(6)
Evoked epileptiform activity	Hippocampus	900 puls at 1 Hz for 15 min	Suppression	(55)
4-aminopyridine	CA1-entorhinal cortex	0.25–1.5 Hz	Suppression	(56)
Mg ²⁺ -free medium	Hippocampus mossy fibers	20–50 s	Suppression	(57)
Tetanic stimulation		0.5 Hz		
4-aminopyridine	Hippocampus-entorhinal cortex	0.5_1 Hz	Suppression	(58)
4-aminopyridine	Hippocampus-entorhinal cortex	1 Hz	Suppression	(59)
Mg ²⁺ -free medium	Hippocampus	1 Hz	Suppression	(60)
Bicuculline	Neocortical brain	0.1- to 5-Hz	Suppression	(61)
Bicuculline		5–30 min		
Bicuculline	Neocortex	0.1- to 5-Hz	Prevention	(61)
Mg ²⁺ -free medium		5–30 min		
4-aminopyridine	Insular cortex	0.2, 0.5, 1 Hz	Suppression	(62)
4-aminopyridine	ventral	1Hz	Suppression	(63)
	Hippocampal commissure	15 min		
4-aminopyridine	Lateral nucleus of the amygdala	1 Hz	Suppression	(64)

blocks limbic discharge generation; however, they proposed that repetitive stimulation may also increase GABA release, which activates presynaptic GABA_B receptors, lead to glutamate release reduction (48). Several mechanisms may contribute to this activity-dependent effect, including the activation of presynaptic metabotropic receptors, which inhibit glutamate release (49), or NMDA transmission is reduced by extracellular alkalization (50). Additionally, it's noteworthy that the stimulus frequency that effectively inhibits ictal discharges is nearly the same as the frequency that elicits long-term depressions of synaptic transmission in the hippocampal formation (51).

Applying repetitive electrical stimulation (1 Hz) delivered approximately inhibited the generation of ictal activity induced by 4-aminopyridine (4AP) or magnesium-free hippocampus-entorhinal cortex slices. Repeating stimuli may not only initiate ictal discharges within the entorhinal cortex network but also have an impact on the propagation of those discharges to the hippocampus (52). In neocortical brain slices that were induced with either bicuculline or magnesium-free extracellular solution, continuous low-frequency stimulation and short trains of high-frequency stimulation were evaluated to determine the effect of the stimulus on epileptiform discharges. Interictal and seizure-like discharges were inhibited by continuous low-frequency stimulation (0.1 to 5 Hz for 5 to 30 minutes) in a way that depended on the frequency, distance, and intensity. The stimulation electrode was located within 1 mm of the epileptic focus, thereby eliminating seizure-like events. Therefore, recurrent stimulation may affect ictal discharge initiation within the entorhinal cortical network as well as their transmission to the hippocampus. As a result, they suggest that consistent electrical stimulation might inhibit GABA_A receptor-mediated synchronization, which is linked to marked elevations of extracellular potassium that can cause ictal discharges in the 4AP *in vitro* model. This would prevent the entorhinal cortex from producing ictal responses. The inhibition of ictogenesis by CA3-driven interictal activity has been

demonstrated in mouse slices treated with 4AP (52, 53).

However, by long-term depressing synaptic transmission within the limbic networks in slice preparation, low-frequency stimulation may prevent ictogenesis. Low-frequency stimulation of the hippocampus causes long-term depression. Ghasemi et al. applied different patterns of LFS to CA1 hippocampal rat slice and declared LFS (1 Hz) 15 min suppressed epileptiform activity induction. Also they showed mGluR1 and mGluR5 had a role in the inhibitory effect of LFS so that blockage of these receptors by their antagonist led to a pronounced reduction of LFS action. Their results confirmed that this effect of mGluRs is mediated by PKC signaling (54-57).

Application of LFS (1 Hz) to the lateral nucleus of the amygdala on ictal-like epileptiform discharges induced by 4-AP in the perirhinal cortex led to the elimination of ictal discharges in a rat brain slice. As a result, epileptiform response latency increased. After that, it was discovered that pharmacologically inhibiting GABA_B receptors decreased the regulation of ictal activity by 1-Hz stimulation, and the accompanying latency increased (58).

On the other hand, the anti-epileptiform activity effects of LFS are mediated by well-known neuromodulators such as norepinephrine. In this regard, studies have shown that anti-epileptiform activity effects of LFS require the activity of receptors, so that alpha-adrenergic receptors antagonist inhibited LFS action on rat hippocampal brain slices (59-61).

By applying bicuculline methiodide to rat hippocampal slices, in the same preparation, Albensi et al. induced epileptiform activity for comparison of low (1 Hz) and high (100 Hz) frequency stimulations. They found that orthodromic stimulation of Schaffer collaterals for 10 minutes diminished normal responses' amplitude and declined epileptiform activity. The stimulus at 1 Hz induced a gradual but persistent suppression, whereas the stimulus at 100 Hz produced a rapid suppression; however, the stimulation at 100 Hz was transient. An NMDA antagonist, D-2-amino-5-phosphonopentanoate (AP5), reversed

the antiepileptic effects of 1 Hz stimulation, and long-term depression may be responsible for suppression by low-frequency stimulation (62).

HFS effect on epileptiform activity

In order to control the aberrant neural activity linked to seizure, and movement problems, high-frequency stimulation (>10 Hz) is performed. However, the mechanisms behind its therapeutic effects remain unknown. In the early years of the study, the effects of electric current on brain sections were primarily limited to the neuronal response. In 1984 Bawin et al. assessed the influence of extracellular sinusoidal electric fields on the amplitude of population spikes evoked by single test pulses in excitatory pathways to CA1 pyramidal neurons in rat hippocampal slices. Brief stimulation (5-30 s) with both 5 and 60 Hz fields (20-70 mV/cm_{p-p} in the perfusing solution) often produced a long-term increase (longer than 10 min) in the population spike. Fields at 60 Hz, but not at 5 Hz, also induced short-term depression (1-6 min) or transient post-field excitation (15-30s). Prolonged stimulation (3 min) emphasized this frequency-dependent response: fields at 5 Hz induced long-lasting potentiation while fields at 60 Hz always resulted in progressive depression persisting for a few minutes after stimulation. During and following 3 min field stimulation at either frequency, antidromic responses were depressed (0.2 mM Ca²⁺, 4mM Mg²⁺), which might be the result of failing calcium mechanisms. There was no direct link between the frequency of the sine wave and the potential evoked by synaptic or antidromic stimulation, which suggests that the fields are not directly polarizing the membrane. EEG-like fields appear to play a functional role in hippocampal excitability, according to experimental evidence (63). In a different investigation, Bawin et al. used penicillin concentrations of 0, 0.25, 1.5, or 3 mM to induce epileptiform activity in rat hippocampus slices. The slices were then briefly subjected to external sinusoidal electric fields (20 s, 5 and 60 Hz, 20-40 mV/cm in tissue). In the CA1 cell layer, fields caused long-term (min) variations in population spike amplitudes; epileptiform responses were suppressed strongly while mildly epileptiform and standard responses were potentiated in post-field studies. Evidence suggests that endogenous extracellular fields are involved in the dynamic control of seizures; epileptiform responses were suppressed powerfully while mildly epileptiform and standard responses were potentiated in post-field studies. Evidence suggests that endogenous extracellular fields are involved in the dynamic control of seizures (64). Additionally, by directly hyperpolarizing pyramidal neurons, constant DC electric fields have been applied in vitro to block high-K⁺ and low-Ca²⁺ generated epileptiform bursting. (65, 66). However, the direction of the external electric field in relation to the neuron's dendritic-somatic axis greatly determines the effectiveness of the suppression (67).

According to Bikson et al., high-frequency stimulation causes potassium efflux and depolarization block to suppress epileptiform activity. Using large parallel wire 'field' electrodes (which uniformly stimulate the entire encom-

passed brain tissue), they demonstrated that 50 Hz continuous sinusoidal electric fields suppress epileptiform activity In vitro (68). It is generally known that extracellular potassium levels rise in response to high-frequency stimulation (69-71) Moderate increases in extracellular potassium activity are consistent with the effect of subthreshold stimulation on the epileptiform event waveform (72, 73). While small increases in potassium can promote epileptiform activity, large increases can suppress spontaneous bursting (74). An increase in extracellular potassium is anticipated to depolarize cells and may cause epileptiform activity to be disrupted by a depolarization block (75). Depolarization block is brought on by the tonic inactivation of Na⁺ channels brought on by sustained membrane depolarization, which prevents the initiation of action potentials (76). We discovered that depolarization of > 20 mV was sufficient to cause a depolarization block, which is consistent with modeling studies (72, 77). When suprathreshold sinusoidal electric fields were applied, the neurons became depolarized above this point, which stopped them from firing action potentials.

Previous studies have demonstrated that epileptiform activity may be suppressed in vitro by uniform electric fields with either AC (continuous sinusoidal) or DC waveforms. Continuous sinusoidal stimulation, sinusoidal stimulation with a 50% duty cycle, and pulsed stimulation with a low duty cycle (1.68%) (120 microseconds, 140 Hz) were all successfully able to completely suppress spontaneous low-Ca²⁺ epileptiform activity. With uniform or localized fields, continuous sinusoidal stimulation could also entirely block epileptiform activity produced by picrotoxin and high K⁺. The monopolar electrode's suppression was restricted to the area near the stimulation electrode. The impacts of AC stimulation were not orientation-specific, as established by potassium concentration and transmembrane potential measurements, and they included an increase in extracellular potassium concentration and a block of neuronal depolarization. While being orientation-selective and blocking activity by membrane hyperpolarization, DC stimulation demonstrated a lower threshold for suppression (78).

Closed-loop HFS application in neocortical brain slices treated with either bicuculline or magnesium-free extracellular solution has antiepileptic effects probably by short-term synaptic depression of excitatory neurotransmission, according to Schiller et al. High-frequency stimulation's ability to treat epilepsy was influenced by its duration, frequency, and intensity but not by the cortical layer stimulated or how near the epileptic spot the stimulating electrode was located (79). Up to 3 s but no longer the dependency on stimulation duration was seen, and up to 50 Hz but no longer the dependence on stimulation frequency. It is crucial to emphasize that this study was the first to investigate whether high-frequency trains may end seizure-like events In vitro before they had fully developed. Their research as a whole revealed that the antiepileptic effects of electrical stimulation were caused by the suppression of excitatory neurotransmission, although high-frequency stimulation may also be partially caused by the reduction of excitability. Their study demonstrates

Table 2. HFS effect on epileptiform activity

Epileptiform activity type	Area	HFS criteria	Effect	Reference
Zero-Ca ²⁺ Low-Ca ²⁺ Picrotoxin High-K ⁺	Hippocampus	Sinusoidal 20-50 Hz	Suppression	(70)
Mg ²⁺ -free Bicucullin	Neocortical brain slice	(1–5 s of 25 to 200-Hz stimulation)	Suppression	(61)
High-K ⁺ and picrotoxin	Hippocampus	Sinusoidal 50 Hz	Suppression	(83)
Low-Mg ²⁺	Hippocampus	130Hz	Suppression	(81)
Low-Ca ²⁺ picrotoxin- and high-K ⁺	Hippocampus	140 Hz	Suppression	(80)

for the first time that glutaminergic synaptic depression primarily mediates the antiepileptic effects of stimulation. They found that electrical stimulation marked depressed EPSPs. However, other pre and postsynaptic processes, such as desensitization of AMPA receptors, glutamate receptor saturation, inactivation of presynaptic voltage-gated calcium channels, and activation of presynaptic metabotropic glutamate receptors, likely also contribute to synaptic depression.

In a study by Zheng et al., epileptiform discharges in isolated hippocampal slices rats were induced by low-Mg²⁺ artificial cerebrospinal fluid and electrical stimulation on the CA3 using concentric bipolar electrodes (square wave, 900 pulses, 50 % duty cycle, 130 Hz) and epileptiform discharges of hippocampal neurons recorded by multi-electrode arrays. They found that HFS increases inter-ictal discharge frequency and decreases ictal discharge duration. However, the HFS did not affect the slices with 10 μmol/L bicuculline, which means that HFS inhibits epileptiform discharges by activating GABA_A receptors and inhibits low-Mg²⁺-induced epileptiform discharges in slices (80). According to this finding, HFS in the hippocampus lowers the excitability of abnormal pathways and increases the likelihood that inhibitory effects would occur, hence lowering abnormal discharges. GABA content increased during 130 Hz HFS and persisted at a high level for more than an hour following the termination of stimulation (81). In Table 2 we briefly review the HFS effect on epileptiform activity.

Discussion

There is a rapidly developmental set of neurostimulation approaches to treat drug-resistant epilepsy, using different stimulation sites, stimulation parameters, and intervention timing. Here we reviewed an in vitro brain slice preparation to compare the effects of low and high-frequency stimulation on epileptiform activity. The main results of our study can be summarized as follows. The onset of inhibition at 1 Hz was gradual, but the onset of inhibition at 100 Hz was rapid. However, the effects of 100 Hz stimulation were short-lived. Furthermore, 1 Hz appeared to be effective in the long-term suppression of evoked and spontaneous epileptiform activity.

Many studies indicated that HFS is an effective therapeutic method both at the site of seizure generation and at a remote site of neuromodulation. However, the underlying mechanisms are still unknown. It has been suggested that the disruption of epileptic activity by applying HFS to thalamic targets is due to the desynchronization of the

epileptogenic network (82). Furthermore, HFS application produces increased extracellular potassium, which results in decreased neuronal activity (83). LFS may also induce long-lasting hyperpolarizing potentials in pyramidal cells (84) and chronic LFS may lead to tissue remodeling (85). Many factors, including neurotransmitters (glutamate and GABA), altered regulation of glutamate transporters, abnormal transcription of GABA and AMPA, and synaptic reorganization, might be related to the anti-seizure effects of the DBS (86, 87). Under physiological conditions, a relatively low percentage of CA3 pyramidal cells are known to form recurrent excitatory connections.

Conclusion

DBS has been used as an alternative treatment strategy for medically refractory epilepsy. Due to the lower current injection into brain tissue, LFS has several advantages over HFS in terms of improving patient safety and increasing pulse generator battery life. There are various hypotheses on the inhibition of electrical stimulation: First, electrical stimulation causes neuronal depolarization and the release of GABA_A in downstream neuronal nuclei, which inhibits neuronal firing. Second, electrical stimulation inhibits the nervous tissue downstream of the stimulation site by preventing the passage of nervous activity from the upstream to the downstream of the axon. Third, electrical stimulation inhibits all brain tissues in close proximity to the stimulating electrode, including axons and cell bodies, and this inhibitory effect weakens with distance.

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Author Contributions

Study conception and design: N.A, M.Kh and M.J; data collection: N.A, M.Z, F.G, N.Am and M.Kh; draft manuscript preparation: N.A, M.Z, M.Kh, F.K, S.M, F.G and Z.Gh. All authors reviewed the results and approved the final version of the manuscript.

Conflict of Interests

The authors declare that they have no competing interests.

References

1. Aghdash SN. Herbal medicine in the treatment of epilepsy. *Curr Drug Targets*. 2021;22(3):356-67.

2. Didato G, Chiesa V, Losito E, Amorim Leite R, Abel TJFiN. Complex Scenarios of Drug-Resistant Epilepsies: Diagnostic Challenges and Novel Therapeutic Options. *Front Mater.* 2022. p. 908163.
3. Vetkas A, Fomenko A, Germann J, Sarica C, Iorio-Morin C, Samuel N, et al. Deep brain stimulation targets in epilepsy: Systematic review and meta-analysis of anterior and centromedian thalamic nuclei and hippocampus. *Epilepsia.* 2022;63(3):513-24.
4. Perez-Malagon CD, Lopez-Gonzalez MA. Epilepsy and Deep Brain Stimulation of Anterior Thalamic Nucleus. *Cureus.* 2021;13(9):e18199.
5. Ryvlin P, Rheims S, Hirsch LJ, Sokolov A, Jehi L. Neuromodulation in epilepsy: state-of-the-art approved therapies. *Lancet Neurol.* 2021;20(12):1038-47.
6. Juhász G, Mittli D, Tukacs V, Kékesi KA. *Electrophysiology and Single Cells. Single Cell 'Omics of Neuronal Cells: Springer; 2022. p. 251-72.*
7. Salanova V, Witt T, Worth R, Henry TR, Gross RE, Nazzaro JM, et al. Long-term efficacy and safety of thalamic stimulation for drug-resistant partial epilepsy. *Neurology.* 2015;84(10):1017-25.
8. Cukiert A, Cukiert CM, Burattini JA, Mariani PP. Long-term seizure outcome during continuous bipolar hippocampal deep brain stimulation in patients with temporal lobe epilepsy with or without mesial temporal sclerosis: An observational, open-label study. *Epilepsia.* 2021;62(1):190-7.
9. Bragdon AC, Kojima H, Wilson WA. Suppression of interictal bursting in hippocampus unleashes seizures in entorhinal cortex: a proepileptic effect of lowering [K⁺]_o and raising [Ca²⁺]_o. *Brain Res.* 1992;590(1-2):128-35.
10. Jerger K, Schiff SJ. Periodic pacing an in vitro epileptic focus. *J Neurophysiol.* 1995;73(2):876-9.
11. Dulla CG, Janigro D, Jiruska P, Raimondo JV, Ikeda A, Lin CCK, et al. How do we use in vitro models to understand epileptiform and ictal activity? A report of the TASK 1-WG 4 group of the ILAE/AES Joint Translational Task Force. *Epilepsia Open.* 2018;3(4):460-73.
12. Wong M. Epilepsy in a dish: an in vitro model of epileptogenesis. *Epilepsy Curr.* 2011;11(5):153-4.
13. Sajadian A, Esteghamat S, Karimzadeh F, Eshaghabadi A, Sieg F, Speckmann EJ, et al. Anticonvulsant effect of neural regeneration peptide 2945 on pentylenetetrazol-induced seizures in rats. *Neuropeptides.* 2015;49:15-23.
14. Dulla CG, Janigro D, Jiruska P, Raimondo JV, Ikeda A, Lin CK, et al. How do we use in vitro models to understand epileptiform and ictal activity? A report of the TASK1-WG4 group of the ILAE/AES Joint Translational Task Force. *Epilepsia Open.* 2018;3(4):460-73.
15. Aghdash SN, Foroughi G. Chemical Kindling as an Experimental Model to Assess the Conventional Drugs in the Treatment of post-Traumatic Epilepsy. *CNS Neurol Disord Drug targets.* 2022.
16. Toprani S, Durand DM. Fiber tract stimulation can reduce epileptiform activity in an in-vitro bilateral hippocampal slice preparation. *Exp Neurol.* 2013;240:28-43.
17. Isaev D, Isaeva E, Khazipov R, Holmes GL. Anticonvulsant action of GABA in the high potassium-low magnesium model of ictogenesis in the neonatal rat hippocampus in vivo and in vitro. *J Neurophysiol.* 2005;94(4):2987-92.
18. Toprani S, Durand DMJEn. Fiber tract stimulation can reduce epileptiform activity in an in-vitro bilateral hippocampal slice preparation. *Exp Neurol.* 2013;240:28-43.
19. Li CL. Cortical intracellular potentials and their responses to strychnine. *J Neurophysiol.* 1959;22(4):436-50.
20. Li CL, Mc IH. Maintenance of resting membrane potentials in slices of mammalian cerebral cortex and other tissues in vitro. *J Physiol.* 1957;139(2):178-90.
21. Schwartzkroin PA, Prince DA. Penicillin-induced epileptiform activity in the hippocampal in vitro preparation. *Ann Neurol.* 1977;1(5):463-9.
22. Nicoll RA, Alger BE. A simple chamber for recording from submerged brain slices. *J Neurosci Methods.* 1981;4(2):153-6.
23. MacDonald RL, Barker JL. Pentylenetetrazol and penicillin are selective antagonists of GABA-mediated post-synaptic inhibition in cultured mammalian neurones. *Nature.* 1977;267(5613):720-1.
24. Uva L, Librizzi L, Marchi N, Noe F, Bongiovanni R, Vezzani A, et al. Acute induction of epileptiform discharges by pilocarpine in the in vitro isolated guinea-pig brain requires enhancement of blood-brain barrier permeability. *Neuroscience.* 2008;151(1):303-12.
25. Nagao T, Alonso A, Avoli M. Epileptiform activity induced by pilocarpine in the rat hippocampal-entorhinal slice preparation. *Neuroscience.* 1996;72(2):399-408.
26. Traynelis SF, Dingledine R. Potassium-induced spontaneous electrographic seizures in the rat hippocampal slice. *J Neurophysiol.* 1988;59(1):259-76.
27. NAMVAR AS, MIRZAE R. Study of Anticonvulsant Effects of Aqueous Extract of Thymus Vulgaris on Chemical Kindling in Male Mice. *J Sabzevar Univ Med Sci.* 2015.
28. Morris ME, Obrocea GV, Avoli M. Extracellular K⁺ accumulations and synchronous GABA-mediated potentials evoked by 4-aminopyridine in the adult rat hippocampus. *Exp Brain Res.* 1996;109(1):71-82.
29. Viitanen T, Ruusuvoori E, Kaila K, Voipio J. The K⁺-Cl⁻ cotransporter KCC2 promotes GABAergic excitation in the mature rat hippocampus. *J Physiol.* 2010;588(Pt 9):1527-40.
30. Avoli M, de Curtis M. GABAergic synchronization in the limbic system and its role in the generation of epileptiform activity. *Prog Neurobiol.* 2011;95(2):104-32.
31. Avoli M, D'Antuono M, Louvel J, Köhling R, Biagini G, Pumain R, et al. Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. *Prog Neurobiol.* 2002;68(3):167-207.
32. Avoli M, Barbarosie M, Lücke A, Nagao T, Lopantsev V, Köhling R. Synchronous GABA-mediated potentials and epileptiform discharges in the rat limbic system in vitro. *J Neurosci.* 1996;16(12):3912-24.
33. Perreault P, Avoli M. 4-aminopyridine-induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. *J Neurosci.* 1992;12(1):104-15.
34. Mody I, Lambert J, Heinemann U. Low extracellular magnesium induces epileptiform activity and spreading depression in rat hippocampal slices. *J Neurophysiol.* 1987;57(3):869-88.
35. Anderson WW, Lewis DV, Swartzwelder HS, Wilson WA. Magnesium-free medium activates seizure-like events in the rat hippocampal slice. *Brain Res.* 1986;398(1):215-9.
36. Avoli M, De Curtis M, Gnatkovsky V, Gotman J, Köhling R, Lévesque M, et al. Specific imbalance of excitatory/inhibitory signaling establishes seizure onset pattern in temporal lobe epilepsy. *J Neurophysiol.* 2016;115(6):3229-37.
37. Yaari Y, Konnerth A, Heinemann U. Nonsynaptic epileptogenesis in the mammalian hippocampus in vitro. II. Role of extracellular potassium. *J Neurophysiol.* 1986;56(2):424-38.
38. Haas H, Jefferys J. Low-calcium field burst discharges of CA1 pyramidal neurones in rat hippocampal slices. *J Physiol.* 1984;354(1):185-201.
39. Krnjević K, Morris ME, Reiffenstein RJ. Changes in extracellular Ca²⁺ and K⁺ activity accompanying hippocampal discharges. *Can J Physiol Pharmacol.* 1980;58(5):579-82.
40. McLaughlin S, Szabo G, Eisenman G. Divalent ions and the surface potential of charged phospholipid membranes. *J Gen Physiol.* 1971;58(6):667-87.
41. Taylor CP, Dudek FE. Synchronous neural afterdischarges in rat hippocampal slices without active chemical synapses. *Science.* 1982;218(4574):810-2.
42. Feng Z, Durand DM. Low-calcium epileptiform activity in the hippocampus in vivo. *J Neurophysiol.* 2003;90(4):2253-60.
43. Stasheff SF, Anderson WW, Clark S, Wilson WA. NMDA antagonists differentiate epileptogenesis from seizure expression in an in vitro model. *Science.* 1989;245(4918):648-51.
44. Bragdon AC, Kojima H, Wilson WAJBr. Suppression of interictal bursting in hippocampus unleashes seizures in entorhinal cortex: a proepileptic effect of lowering [K⁺]_o and raising [Ca²⁺]_o. *Brain Res.* 1992;590(1-2):128-35.
45. Jerger K, Schiff SJJJoN. Periodic pacing an in vitro epileptic focus. *J Neurophysiol.* 1995;73(2):876-9.
46. Barbarosie M, Avoli MJJoN. CA3-driven hippocampal-entorhinal loop controls rather than sustains in vitro limbic seizures. *J Neurosci.* 1997;17(23):9308-14.
47. Khosravani H, Carlen PL, Velazquez JLPJBJ. The control of seizure-like activity in the rat hippocampal slice. *Biophys J.* 2003;84(1):687-95.
48. Benini R, D'antuono M, Pralong E, Avoli MJN. Involvement of amygdala networks in epileptiform synchronization in vitro. *Neuroscience.* 2003;120(1):75-84.
49. Scanziani M, Salin PA, Vogt KE, Malenka RC, Nicoll RAJN. Use-dependent increases in glutamate concentration activate presynaptic

- metabotropic glutamate receptors. *Nature*. 1997;385(6617):630-4.
50. De Curtis M, Manfredi A, Biella GJJoN. Activity-dependent pH shifts and periodic recurrence of spontaneous interictal spikes in a model of focal epileptogenesis. *J Neurosci*. 1998;18(18):7543-51.
 51. Kimura A, Pavlides CJJon. Long-term potentiation/depotentialization are accompanied by complex changes in spontaneous unit activity in the hippocampus. *J Neurophysiol*. 2000;84(4):1894-906.
 52. D'Arcangelo G, Panuccio G, Tancredi V, Avoli MJNod. Repetitive low-frequency stimulation reduces epileptiform synchronization in limbic neuronal networks. *Neurobiol Dis*. 2005;19(1-2):119-28.
 53. Barbarosie M, Louvel J, D'Antuono M, Kurcewicz I, Avoli MJE. Masking synchronous GABA-mediated potentials controls limbic seizures. *Epilepsia*. 2002;43(12):1469-79.
 54. Ghasemi Z, Naderi N, Shojaei A, Ahmadirad N, Raoufy MR, Mirnajafi-Zadeh J. Low Frequency Electrical Stimulation Attenuated The Epileptiform Activity-Induced Changes in Action Potential Features in Hippocampal CA1 Pyramidal Neurons. *Cell J*. 2018;20(3):355-60.
 55. Ghasemi Z, Naderi N, Shojaei A, Raoufy MR, Ahmadirad N, Barkley V, et al. The inhibitory effect of different patterns of low frequency stimulation on neuronal firing following epileptiform activity in rat hippocampal slices. *Brain Res*. 2019;1706:184-95.
 56. Ghasemi Z, Naderi N, Shojaei A, Raoufy MR, Ahmadirad N, Barkley V, et al. Group I metabotropic glutamate receptors contribute to the antiepileptic effect of electrical stimulation in hippocampal CA1 pyramidal neurons. *Epilepsy Res*. 2021;178:106821.
 57. Ghasemi Z, Naderi N, Shojaei A, Raoufy MR, Ahmadirad N, Mirnajafi-Zadeh J. Effect of low-frequency electrical stimulation on the high-K⁺-induced neuronal hyperexcitability in rat hippocampal slices. *Neuroscience*. 2018;369:87-96.
 58. Kano T, Inaba Y, D'Antuono M, Biagini G, Levésque M, Avoli MJJoN. Blockade of in vitro ictogenesis by low-frequency stimulation coincides with increased epileptiform response latency. *J Neurophysiol*. 2015;114(1):21-8.
 59. Ahmadirad N, Fathollahi Y, Janahmadi M, Shojaei A, Ghasemi Z, Barkley V, et al. Low-Frequency Electrical Stimulation Reduces the Impairment in Synaptic Plasticity Following Epileptiform Activity in Rat Hippocampal Slices through α_1 , But Not α_2 , Adrenergic Receptors. *Neuroscience*. 2019;406:176-85.
 60. Ahmadirad N, Fathollahi Y, Janahmadi M, Ghasemi Z, Shojaei A, Rezaei M, et al. The role of α adrenergic receptors in mediating the inhibitory effect of electrical brain stimulation on epileptiform activity in rat hippocampal slices. *Brain Res*. 2021;1765:147492.
 61. Rezaei M, Ahmadirad N, Ghasemi Z, Shojaei A, Raoufy MR, Barkley V, et al. Alpha adrenergic receptors have role in the inhibitory effect of electrical low frequency stimulation on epileptiform activity in rats. *Int J Neurosci*. 2021;1-9.
 62. Albensi BC, Ata G, Schmidt E, Waterman JD, Janigro DJBr. Activation of long-term synaptic plasticity causes suppression of epileptiform activity in rat hippocampal slices. *Brain Res*. 2004;998(1):56-64.
 63. Bawin SM, Sheppard AR, Mahoney MD, Adey WR. Influences of sinusoidal electric fields on excitability in the rat hippocampal slice. *Brain Res*. 1984;323(2):227-37.
 64. Bawin SM, Abu-Assal ML, Sheppard AR, Mahoney MD, Adey WR. Long-term effects of sinusoidal extracellular electric fields in penicillin-treated rat hippocampal slices. *Brain Res*. 1986;399(1):194-9.
 65. Gluckman BJ, Neel EJ, Netoff TI, Ditto WL, Spano ML, Schiff SJ. Electric field suppression of epileptiform activity in hippocampal slices. *J Neurophysiol*. 1996;76(6):4202-5.
 66. Warren RJ, Durand DM. Effects of applied currents on spontaneous epileptiform activity induced by low calcium in the rat hippocampus. *Brain Res*. 1998;806(2):186-95.
 67. Ghai RS, Bikson M, Durand DM. Effects of applied electric fields on low-calcium epileptiform activity in the CA1 region of rat hippocampal slices. *J Neurophysiol*. 2000;84(1):274-80.
 68. Bikson M, Lian J, Hahn PJ, Stacey WC, Sciortino C, Durand DM. Suppression of epileptiform activity by high frequency sinusoidal fields in rat hippocampal slices. *J Physiol*. 2001;531(Pt 1):181-91.
 69. Gardner-Medwin AR. A study of the mechanisms by which potassium moves through brain tissue in the rat. *J Physiol*. 1983;335:353-74.
 70. Bawin SM, Abu-Assal ML, Sheppard AR, Mahoney MD, Adey WR. Long-term effects of sinusoidal extracellular electric fields in penicillin-treated rat hippocampal slices. *Brain Res*. 1986;399(1):194-9.
 71. Kaila K, Lamsa K, Smirnov S, Taira T, Voipio JJJoN. Long-lasting GABA-mediated depolarization evoked by high-frequency stimulation in pyramidal neurons of rat hippocampal slice is attributable to a network-driven, bicarbonate-dependent K⁺ transient. *J Neurosci*. 1997;17(20):7662-72.
 72. Pinsky PF, Rinzel JJocn. Intrinsic and network rhythmogenesis in a reduced Traub model for CA3 neurons. *J Comput Neurosci*. 1994;1(1):39-60.
 73. Bikson M, Ghai RS, Baraban SC, Durand DMJJon. Modulation of burst frequency, duration, and amplitude in the zero-Ca²⁺ model of epileptiform activity. *J Neurophysiol*. 1999;82(5):2262-70.
 74. Rutecki PA, Lebeda FJ, Johnston DJJon. Epileptiform activity induced by changes in extracellular potassium in hippocampus. *J Neurophysiol*. 1985;54(5):1363-74.
 75. Poolos N, Mauk M, Kocsis JJJon. Activity-evoked increases in extracellular potassium modulate presynaptic excitability in the CA1 region of the hippocampus. *J Neurophysiol*. 1987;58(2):404-16.
 76. Hille B. Ionic channels in excitable membranes. Current problems and biophysical approaches. *Biophys J*. 1978;22(2):283-94.
 77. Traub RD, Wong RK, Miles R, Michelson HJJon. A model of a CA3 hippocampal pyramidal neuron incorporating voltage-clamp data on intrinsic conductances. *J Neurophysiol*. 1991;66(2):635-50.
 78. Lian J, Bikson M, Sciortino C, Stacey WC, Durand DM. Local suppression of epileptiform activity by electrical stimulation in rat hippocampus in vitro. *J Physiol*. 2003;547(Pt 2):427-34.
 79. Schiller Y, Bankirer Y. Cellular mechanisms underlying antiepileptic effects of low- and high-frequency electrical stimulation in acute epilepsy in neocortical brain slices in vitro. *J Neurophysiol*. 2007;97(3):1887-902.
 80. Zheng Y, Zhang K, Dong L, Tian C. Study on the mechanism of high-frequency stimulation inhibiting low-Mg²⁺-induced epileptiform discharges in juvenile rat hippocampal slices. *Brain Res Bull*. 2020;165:1-13.
 81. Luna-Munguía H, Meneses A, Peña-Ortega F, Gaona A, Rocha L. Effects of hippocampal high-frequency electrical stimulation in memory formation and their association with amino acid tissue content and release in normal rats. *Hippocampus*. 2012;22(1):98-105.
 82. Yu T, Wang X, Li Y, Zhang G, Worrell G, Chauvel P, et al. High-frequency stimulation of anterior nucleus of thalamus desynchronizes epileptic network in humans. *Brain*. 2018;141(9):2631-43.
 83. Avoli M, De Curtis M, Köhling R. Does interictal synchronization influence ictogenesis? *Neuropharmacology*. 2013;69:37-44.
 84. Toprani S, Durand DM. Long-lasting hyperpolarization underlies seizure reduction by low frequency deep brain electrical stimulation. *J Neurophysiol*. 2013;591(22):5765-90.
 85. Cavus I, Pan JW, Hetherington HP, Abi-Saab W, Zaveri HP, Vives KP, et al. Decreased hippocampal volume on MRI is associated with increased extracellular glutamate in epilepsy patients. *Epilepsia*. 2008;49(8):1358-66.
 86. Wang Y, Chen Z. An update for epilepsy research and antiepileptic drug development: Toward precise circuit therapy. *Pharmacol Pharmacol Ther*. 2019;201:77-93.
 87. Rocha L. Interaction between electrical modulation of the brain and pharmacotherapy to control pharmacoresistant epilepsy. *Pharmacol Ther*. 2013;138(2):211-28.