Update on the mechanisms and roles of high-frequency oscillations in seizures and epileptic disorders

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SUMMARY

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High-frequency oscillations (HFOs) are a type of brain activity that is recorded from brain regions capable of generating seizures. Because of the close association of HFOs with epileptogenic tissue and ictogenesis, understanding their cellular and network mechanisms could provide valuable information about the organization of epileptogenic networks and how seizures emerge from the abnormal activity of these networks. In this review, we summarize the most recent advances in the field of HFOs and provide a critical evaluation of new observations within the context of already established knowledge. Recent improvements in recording technology and the introduction of optogenetics into epilepsy research have intensified experimental work on HFOs. Using advanced computer models, new cellular substrates of epileptic HFOs were identified and the role of specific neuronal subtypes in HFO genesis was determined. Traditionally, the pathogenesis of HFOs was explored mainly in patients with temporal lobe epilepsy and in animal models mimicking this condition. HFOs have also been reported to occur in other epileptic disorders and models such as neocortical epilepsy, genetically determined epilepsies, and infantile spasms, which further support the significance of HFOs in the pathophysiology of epilepsy. It is increasingly recognized that HFOs are generated by multiple mechanisms at both the cellular and network levels. Future studies on HFOs combining novel high-resolution in vivo imaging techniques and precise control of neuronal behavior using optogenetics or chemogenetics will provide evidence about the causal role of HFOs in seizures and epileptogenesis. Detailed understanding of the pathophysiology of HFOs will propel better HFO classification and increase their information yield for clinical and diagnostic purposes.
KEY WORDS: High-frequency oscillations, Epilepsy, Ripples, Fast ripples, Ictogenesis, Epileptogenesis, Seizures, Interneurons, Computer models.

The identification of high-frequency oscillations (HFOs) in epileptogenic tissue is one of the major discoveries in epilepsy research over the past two decades, attracting the attention of clinical and experimental epileptologists worldwide. HFOs refer to distinct types of brain activity occurring in a frequency band ranging from 80 to 600 Hz.

KEY POINTS

- This review examines recent insights into the cellular and network mechanisms of HFOs
- HFOs are likely generated by multiple, possibly not exclusive, mechanisms occurring at the cellular and network level
- Interneurons play a complex role in epileptic HFOs, and their involvement is dependent on the subtype of HFOs
- Cellular mechanisms and features of HFOs associated with seizure initiation are determined by the nature of the seizure-onset type
- Epileptic HFOs are a widely abundant phenomenon that can be observed in various types of epilepsies ranging from temporal lobe epilepsy, posttraumatic epilepsy, to epilepsies occurring during development

HFOs are classified as wave forms with frequencies faster than gamma activity (30–80 Hz), and are somewhat arbitrarily separated into ripples (80–250 Hz) and fast ripples (250–600 Hz).[1,2]

The presence of HFOs between seizures, at seizure onset, and during seizures suggests an inherent relationship between the cellular and network mechanisms of seizures and HFOs. Characterizing the underlying mechanisms of seizures has been notoriously difficult due to technological limitations and the vast spatial and temporal scales involved; however, HFOs occur on a much smaller and experimentally tractable scale, down to 1 mm^3.[3] Because recording technology has improved over the last two decades, much research has focused on the underlying mechanisms of HFOs, both of those associated with normal brain processes[4] as well as with epileptic processes.[1] Understanding the mechanisms of so-called "epileptic" or "pathological" HFOs should lead to a better understanding of the functional organization of epileptogenic tissue and of the abnormal dynamics of epileptic neuronal networks.

In clinical epileptology and particularly in epilepsy surgery, the identification of a close spatial association between the location of HFOs and the epileptogenic zone and/or the seizure-onset zone has led to the incorporation of measurements of HFO properties into presurgical examinations (see the review by Frauscher et al. in this issue of *Epilepsia*[5]). Many epilepsy surgery centers are now equipped with devices and analytical approaches that allow recording of wide-band signals and extraction of relevant information about the spatiotemporal properties of HFOs to better delineate the epileptogenic zone, and are currently performing clinical trials to determine if this information will improve the outcomes of epilepsy surgery. In addition, understanding spatial and temporal relationships of HFOs over long time scales offers the unique opportunity to use them as a clinical marker of epileptogenesis.

Our knowledge of HFO mechanisms has been substantially advanced after the first *High-Frequency Oscillation Workshop* held in Montreal, Quebec, Canada in 2011.[1] The main aim of our review is to provide the latest insights into the mechanisms of HFOs and to explore possible future research directions to address the unresolved aspects of HFOs, which were presented and discussed during the *2nd International Workshop on High Frequency Oscillations in Epilepsy* held in Freiburg, Germany, in 2016. Our review will focus on the following topics: (1) What are the cellular and network mechanisms involved in the genesis of physiologic and pathologic HFOs; (2) What is the role of interneurons in the genesis of pathologic HFOs; (3) What is known about the causal role of HFOs in ictogenesis; and (4) What progress has been made in characterizing HFOs in different experimental models of epilepsy.

CELLULAR MECHANISMS OF EPILEPTIC HFOS

After the initial descriptions of HFOs occurring under physiologic conditions,[6,7] it has later become clear that HFOs are increased in epileptic tissue.[3,8] However, this situation poses an important problem: how can one distinguish between physiologic and pathologic HFOs?[9] One intriguing initial finding was that, in both human conditions and animal models, it appeared that higher frequency HFOs (>250 Hz), that is, fast ripples, were specific to epileptic tissue. These findings provided an important start to the search for HFO mechanisms and for the characterization of their role in epilepsy.

The past two decades have uncovered several key aspects of HFO mechanisms. One clear result is that HFOs are generated by multiple mechanisms such as synchronized inhibitory postsynaptic potentials with sparse pyramidal cell firing[7] or principal cell action potentials.[10–12] It is now considered that pathologic HFOs, whether they be ripples or fast ripples, reflect mainly principal cell action potentials (Fig. 1).[10,13–16] Synchronization of fast firing within the population of interconnected neurons leads to the formation of an episode of high-frequency population spikes, which is extracellularly recorded as an HFO event (Fig. 1A,B). This mechanism requires synchronization on a millisecond time scale, which is achieved via fast synaptic transmission or nonsynaptic mechanisms like gap-junction coupling[17] or ephaptic interactions—a synchronizing mechanism that depends on specific geometric organization and tight cellular arrangement.[18]

The mechanisms establishing the frequency of the oscillation vary. Frequency can be determined purely from the cellular behavior, that is, from the action potential firing rate of the principal cells. These are called "pure" HFOs.[15] However, individual pyramidal cells cannot fire fast enough to produce synchronized oscillations up to 600 Hz, that is, fast ripples. Even in epileptic neurons the rate of action potential
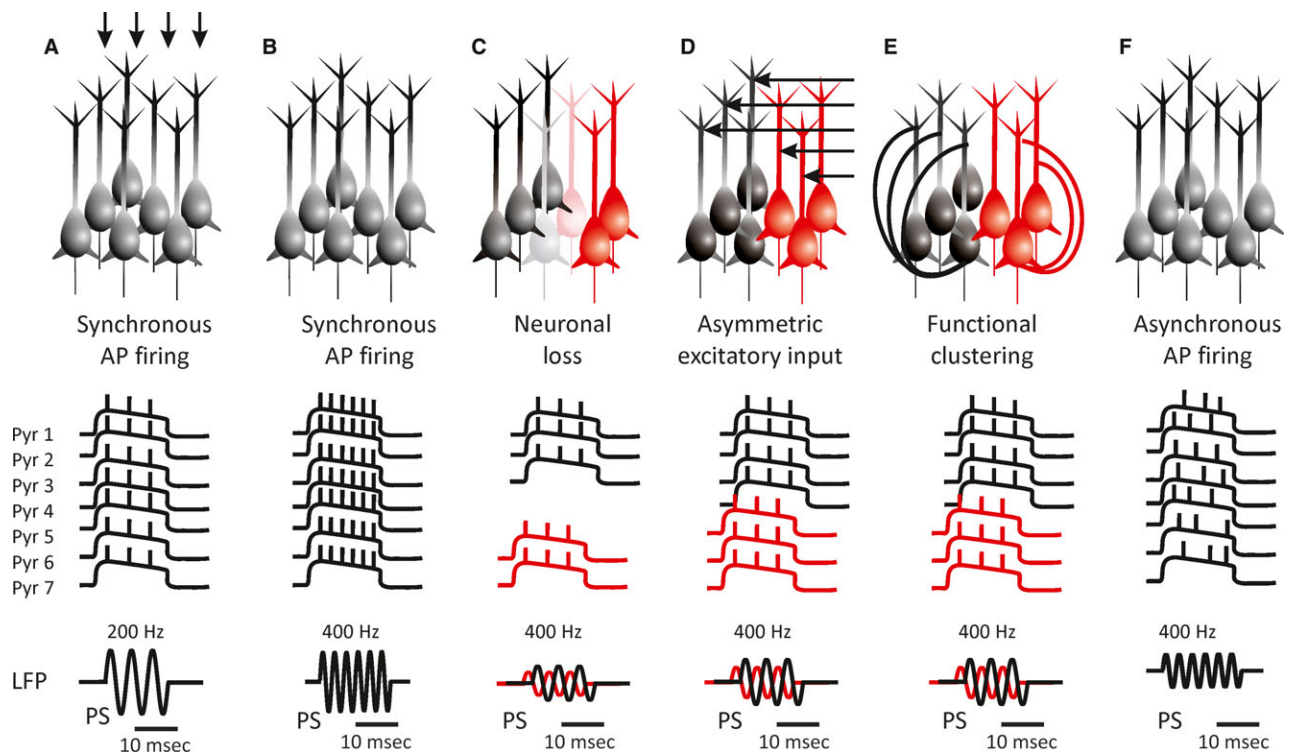
Figure 1.
Cellular and network mechanisms of pathologic HFOs. (**A**) HFOs are generated by synchronous action potential firing of principal cells. An individual cycle of an oscillation is a population spike. The frequency of the oscillation is determined by the frequency of the cellular firing-so called "pure" HFOs. (**B**) Fast ripples, that is, HFOs with a frequency of up to 600 Hz, can be generated, if the cells fire action potentials at the same frequency. Physiologic mechanisms underlying the genesis of action potentials limit the rate of cellular firing. Therefore, this mechanism is least probable. (**C**) Out-of-phase firing between two neuronal populations can result in a doubling of the frequency of the extracellularly recorded "emergent" type of HFOs. In this scenario, the out-of-phase firing is due to cell loss. (**D**) Asymmetric excitatory input or polychronicity due to various lengths of axons can result in functional clustering and out-of-phase firing. (**E**) Morphologic changes, axonal growth and sprouting may result in functional clustering due to the presence of hub neurons. (**F**) Asynchronous firing within an active neuronal population may result in random coincidental firing and the occurrence of fast ripples.
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firing is often limited to <300 Hz.[15] The currently accepted explanation of fast ripple generation—with frequencies beyond the physiologic limits of neuronal firing—is the existence of different subpopulations of synchronized neurons that have a phase delay (Fig. 1C,D).[1,19] Fast ripples may thus represent the net frequency of neuronal populations each oscillating with a lower frequency, and thus they represent an "emergent" HFO.[15,20,21] Several network mechanisms that are responsible for out-of-phase firing were proposed based on experimental evidence or results obtained from computer modeling. Reduced spike time-variability,[14] uncorrelated firing, delayed activation, disconnected neural populations, or complex network connectivity patterns with a high level of clustering due to the presence of hub neurons (Fig. 1E) can all contribute to out-of-phase activation. In temporal lobe epilepsy, the rate of fast ripples in the hippocampus correlates positively with the severity of cell loss[22]; it has been suggested that the loss of neurons can contribute to out-of-phase firing (Fig. 1C),

probably by decreasing the synchronizing effect of ephaptic interactions.[14,22]

Research into the precise mechanisms has been limited by recording technology. Even within the very small scale of epileptic fast ripples in the hippocampus, there are thousands of cells involved, far beyond the resolution provided by modern microelectrode arrays. Recent advances in imaging techniques, such as voltage-sensitive dyes and calcium imaging, have allowed recordings from many cells simultaneously, but they are still unable to function at the fast time scale necessary for HFOs. Thus computational models have been necessary to guide research into HFO mechanisms.[23] Great care must be taken in both the development and interpretation of computational models to ensure that they are physiologically grounded and to validate their findings whenever possible. It is also important to note that any modeling result will be limited by the phenomena included within the model itself. When performed properly, modeling predictions can then guide future experiments as

technology advances. Several models have used this methodologic approach to assess the mechanisms involved in the generation of ripples and fast ripples that could not be tested directly with current technology. Various models of ripples have been proposed and validated experimentally.[1] Fast ripples, with their apparent increased specificity to epileptic tissue, have garnered great interest in investigating mechanisms they might share with seizures. However, fast ripples have been very challenging to explain, even in silico.

Previous modeling work had identified three potential mechanisms of fast ripples: gap junction networks,[24] desynchronous groups of firing cells,[14,15] or activated, but weakly synchronized, pyramidal cells.[13] Other studies have described how physiologic HFOs could transition to epileptic HFOs with pathologies such as loss of inhibition, or increased coupling from gap junctions, or recurrent synapses and increased synaptic activity.[25,26] A recent detailed model, designed to mimic how both pyramidal action potentials and inhibitory postsynaptic potentials (IPSPs) appear on an intracranial recording electrode, demonstrated several important predictions about HFOs.[27] First, at frequencies below 250 Hz, both epileptic and physiologic processes can produce HFOs with identical peak frequencies, suggesting that they cannot be distinguished by frequency alone. However, the conditions that produce epileptic ripple oscillations (disrupted inhibitory coupling) often produce brief instances of fast ripples that emerge spontaneously. Second, they demonstrated mathematically that it is impossible for IPSPs to generate oscillations >250 Hz due to their morphology.

Another important modeling finding is the growing evidence from several groups that fast ripple oscillations are a generic feature of highly active, desynchronized networks.[13,27] These in silico observations are corroborated by the experimental evidence in chronic models of epilepsy and in human epileptic tissue.[15,28,29] These diverse mechanisms lead to a similar conclusion about the relationship between epileptic spikes and epileptic HFOs: large groups of hyperexcitable, hypersynchronized pyramidal cells produce spikes, but if the connectivity of cells is disrupted, then cells would fire out of phase and produce fast ripples.

The findings that have been reviewed earlier should explain how the neuronal network can synchronize and produce HFOs. However, recent work has shown that HFOs can be generated even in the absence of any network connectivity.[27] These results were recently proven analytically,[16] and although they may seem counterintuitive to the neuroscience and HFO community, they are a well-known phenomenon in complex dynamics. Essentially, a synchronous discharge from an ensemble of cells (which results in a local field potential) does not require any network synchrony or coupling at all, only that the neurons be actively firing at similar frequencies (Fig. 1F). The superposition of many neurons firing at a certain frequency, even if they are randomly distributed in phase, will be an oscillation at that frequency. Thus HFOs may simply represent a marker of highly activated neurons, regardless of the underlying structure or mechanism. A similar phenomenon was proposed based on observations in vitro in low-calcium or high-potassium models of seizures, where intense neuronal activity may lead to coincidental firing among cells, which manifests as high-frequency activity in the ripple band.[21]

An interesting result from recent in vitro studies demonstrates the ambiguous behavior of neuronal networks underlying the genesis of HFO under various experimental conditions. For instance, the same neuronal network can have different IPSP and excitatory postsynaptic potentials dynamics during ripple-like activity, depending on extracellular concentration levels of Ca^{2+}.[30] These authors reported that at 3 mM Ca^{2+}, the frequency of the HFO was 126 ± 13 Hz and correlated with coordinated firing of putative pyramidal cells and interneurons; instead, at 1 mM Ca^{2+}, the frequency was slightly higher (200–300 Hz), with an important increase in firing of cells.[30] Similarly, Alvarado-Rojas et al. reported two types of neuronal behavior correlated with similar-appearing HFOs in the human subiculum tissue maintained in vitro.[28] In this case, ripple-like HFOs (100–250 Hz) were produced by either strong rhythmic inhibitory postsynaptic potentials or strong synaptic depolarization with pyramidal cell bursting. Thus in agreement with the modeling predictions, the frequency of the HFOs may not be a valid method to determine the underlying mechanism or epileptogenicity. Similarly, microelectrode recordings from human tissue have shown that putative pyramidal cells and interneurons have complex behaviors, wherein some cells become more or less active and often do not appear to synchronize during HFO occurrence.[28,31] Therefore, cell activity is not necessarily synchronized, but heterogeneous firing is a common behavior for pathologic HFOs. These findings in human networks support the results from in vitro and computational models.

It is well documented that ripples can be either physiologic or pathologic. However, fast ripples were at first thought to be reliable markers of epileptic tissue. It is now clear that the activity in the fast ripple band can accompany cognitive processes in humans.[32] Given the broad range of pathologies, species, and brain regions that produce HFOs,[2,33] it is likely that HFOs are a generic phenomenon of neural networks that have some complex relationship with epileptic processes. Although their value as a potential biomarker is very strong, it is clear that simply focusing on the peak frequency of HFOs may not be sufficient to determine whether they are pathologic.

THE ROLE OF INTERNEURONS IN HFOs

The role of individual interneuronal subtypes has been described in detail in the context of physiologic gamma

oscillations and sharp-wave ripples.[4,34] These oscillations are driven by interneuronal activity and can facilitate temporal coding, fast processing, and flexible routing of neuronal activity, which are necessary for cognition.[35] Such interneuronal activity makes principal cells generate a series of fast inhibitory postsynaptic potentials that are extracellularly recorded as physiologic HFOs (Fig. 2A). Surprisingly, the role of interneurons or individual interneuronal subtypes in pathologic HFOs is not so well defined. HFOs often occur superimposed on interictal epileptiform discharges, which display very complex interneuronal activity and have been evaluated extensively by Karlocai et al. in various in vitro models[36]; these investigators found that although dendritic interneurons and cholecystokinin-positive basket cells increase their firing rate, perisomatic inhibition fails due to depolarization block of parvalbumin-positive neurons. Unfortunately, in their study they did not examine the phase relationship with superimposed HFOs. Recently, Morris et al. evaluated the role of interneurons in the CA3 region during pathologic ripples using brain slices with fluorescently labeled interneurons perfused with high-potassium artificial cerebrospinal fluid.[37] Approximately 42% of interneurons increased firing during individual cycles of ripple oscillations, suggesting that γ-aminobutyric acid (GABA)ergic signaling is preserved on a cycle-by-cycle basis during ripples (Fig. 2B).

Although inhibition may be important for ripples, it seems that fast ripples do not depend on intact fast inhibition. Computational studies revealed that reduction of GABAergic connections from basket cells on pyramidal neurons,[27] or moderate reduction in GABAergic conductance with a simultaneous moderate increase in N-methyl-D-aspartate (NMDA) conductance[13] favored the increased probability of the emergence of fast ripples from ripple activity (Fig. 2C,D). Further support to these experimental observations comes from a model of temporal lobe epilepsy induced by intrahippocampal injection of tetanus toxin.[38] Tetanus toxin leads to complete abolition of fast inhibitory transmission around the site of injection by blocking neurotransmitter release, yet the hippocampus was still able to generate fast ripples.[39] The evidence obtained from all these studies supports the hypothesis that fast ripple generation
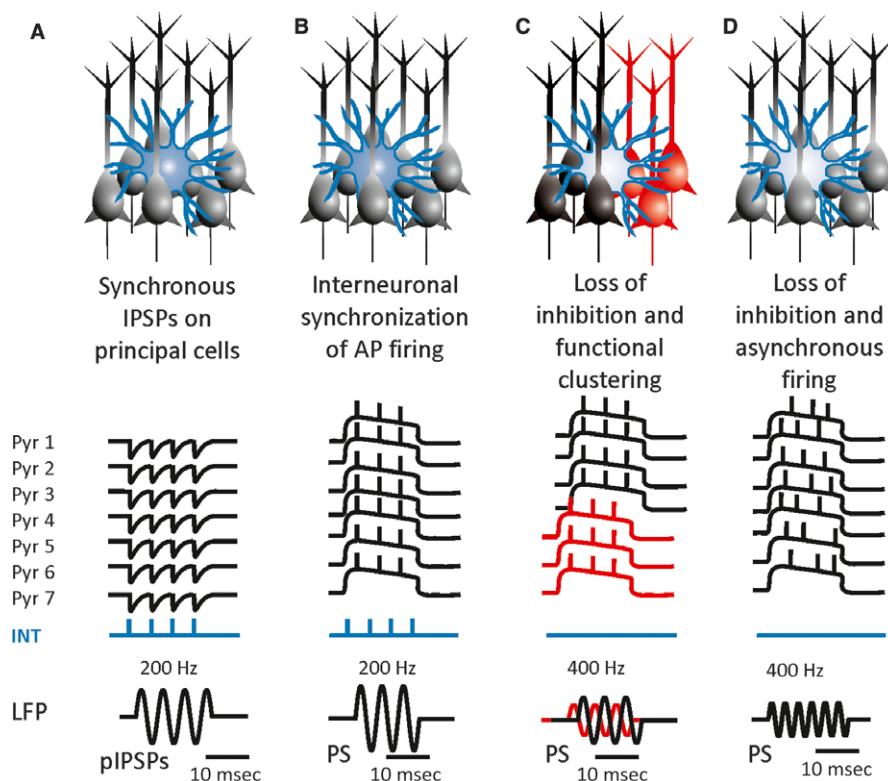

Figure 2.
The role of interneurons in HFOs. (**A**) HFOs may represent extracellularly recorded, synchronous, inhibitory, postsynaptic potentials on the membranes of principal cells. This mechanism underlies physiologic sharp-wave ripples. Pathologically, they may be involved in low-amplitude fast activity ictal onset but usually with frequencies lower than ripples. (**B**) In epileptic ripples, the interneuronal activity and inhibitory postsynaptic potentials control the action potential firing of the active epileptic population, which manifests as pathologic ripples. This mechanism is dependent on intact perisomatic inhibition maintained by basket cells. (**C**) Loss of inhibition may play a role in the pathogenesis of fast ripples. The absence of rhythmogenic fast inhibition may result in functional clustering or asynchronous neuronal firing and the generation of fast ripples (**D**).
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does not require intact inhibition and that fast ripples may mark regions with eroded inhibition; this point may also explain the presence of fast ripples at the onset of some types of focal seizures such as those presenting with hypersynchronous onset (see below).

HFOs in Ictogenesis

HFOs are involved in seizure genesis prior to,[1,21,40,41] and during[42,43] the seizure onset. The precise role of HFOs appears to depend on the type of seizure-onset pattern.[43] Several distinct seizure-onset patterns have been reported,[44] and two of these patterns display clear association with HFOs. The low-voltage fast (LVF) seizure-onset pattern consists of low-voltage beta and gamma oscillations >12 Hz,[45] can be seen in both limbic and neocortical seizures, and can occur during both seizure genesis and spread.[41,44] Inhibitory interneurons play a central role in orchestrating LVF onset.[46–48] Intracellular recordings from slices of entorhinal cortex[49,50] and the guinea pig whole brain preparation[46] have shown that at LVF onset, principal neurons generate robust IPSPs[49] and few action potentials. These events are followed by excitation associated with a dramatic increase in firing rate in principal cells. In support of the fundamental role played by inhibitory interneurons in promoting LVF seizure genesis, optogenetic activation of parvalbumin- or somatostatin inhibitory cells in the mouse entorhinal cortex slice bathed in 4-aminopyridine triggered LVF-onset seizures.[47] LVF activity is typically associated with the ripple band.[41–43] The likelihood that the superimposed pathologic ripple activity is also generated by inhibitory interneurons is corroborated but optogenetic experiments, which demonstrated that light stimulation of parvalbumin interneurons triggers LVF seizures associated with ripples.[43]

The hypersynchronous (HYP)–onset pattern consists of sharply contoured high-voltage ictal discharges that occur at a frequency of <2 Hz.[45] HYP-onset seizures appear to begin exclusively in limbic structures,[44] and often in the setting of mesial temporal sclerosis.[51,52] Typically, fast ripples precede or accompany HYP discharges.[40,41] From the cellular perspective, HYP onset involves increased principal neuron firing and a progressive weakening of inhibition. Intracellular recordings from principal neurons in the perirhinal cortex during application of 4-aminopyridine have shown that principal neurons generate action potential bursts during the HYP discharge as well as that the postburst hyperpolarization (presumably caused by activation of postsynaptic GABA$_A$ receptors) progressively decreases in amplitude. This phenomenon is accompanied by a gradual positive shift in the reversal potential of the postburst hyperpolarizations along with an increase in the associated transient elevations in $[K^+]_o$.[53] In such a scenario, superimposed epileptic fast ripples are assumed to mirror action-potential firing of principal cells.[3,10,54]

Studies of human seizures using devices capable of resolving multiunit and single unit activity have revealed that within brain regions demonstrating the electroencephalography (EEG) signature of a seizure, two territories can be defined based on characteristic multiunit firing patterns. The relatively small ictal core is defined by high-amplitude EEG discharges corresponding to synchronized, intense multiunit firing bursts, occurring in the context of paroxysmal depolarizing shifts. In the penumbra, dominated by surround inhibition,[55,56] firing is sparse and heterogeneous, unrelated to the ongoing EEG rhythm.[57,58] This distinction may provide a useful framework for interpreting HFO activity detected during seizures.[59]

Trains of high-gamma band phase-locked to the dominant rhythm of ictal discharges can pinpoint the location of the ictal core.[59] The high-gamma band activity is characterized by a delayed onset, several seconds after the start of the seizure, and sustained activity through the remainder of the seizure. In this type of seizure onset, mathematical modeling suggests that the superimposed high-gamma band activity reflects a slightly jittered but otherwise synchronous summation of strong postsynaptic currents generated by paroxysmal depolarization shifts on the membrane of intensely firing principal cells.[60] In the surrounding penumbra, pyramidal cell firing is not sufficiently synchronized to generate a high gamma signature, resulting in an absence of ictal high-gamma activity in this region. Thus the ictal core can be effectively distinguished from the penumbra by the presence of sustained trains of ictal high-gamma activity. This potentially useful EEG interpretation method has been shown to be an effective predictor of surgical outcome, with a trend toward superior performance compared to a predictor based on the seizure-onset zone.[31]

Recently, the relationship between HFOs and the direct current (DC) shift during the seizure onset in patients with implanted electrodes was explored.[61] This study has demonstrated that the seizure onset was accompanied by a negative DC shift and co-localized with the presence of HFOs (from both ripple and fast ripple bands). The DC shift onset slightly preceded the onset of HFOs, and both electrographic phenomena were more spatially limited compared to conventionally defined seizure onset zones.

HFOs in Epileptogenesis

The first study of HFOs during the development of epilepsy was performed in the intrahippocampal kainic acid rat model.[62] In this study, microelectrode recordings showed the appearance of ripple- and fast ripple-frequency HFOs in the dentate gyrus ipsilateral to the injection in rats that subsequently developed spontaneous seizures, whereas HFOs were not found in those kainic acid–treated animals that did not develop epilepsy. In addition, an earlier appearance of HFOs after status epilepticus correlated with a shorter latency and earlier appearance of spontaneous seizures as

well as with a higher rate of seizures per month. These results support the hypothesis that the neuronal disturbances associated with pathologic HFOs play a role in epileptogenesis. Surprisingly, not so many studies have examined this predictive role of HFOs, which—if confirmed in humans—could substantially impact the clinical approach to patients at risk of developing epilepsy after brain trauma, stroke, or other insults.

It has also been reported in this animal model of temporal lobe epilepsy that following the initial pilocarpine-induced status epilepticus, there is a shift in the occurrence of both interictal spikes and HFOs between the hippocampus and the entorhinal cortex at the transition from the latent to the chronic period.[63] In addition, in this study, it was found that fast ripples occurring outside of spikes had higher rates in the entorhinal cortex than in the hippocampus during the latent phase, whereas the opposite occurred during the epileptic phase.[63] This transition may thus reflect the dynamic activity and progressive pathologic reorganization of limbic neuronal networks during epileptogenesis.

Once epilepsy develops, HFOs may also be used as a marker of disease activity. Both experimental and clinical studies have shown that the incidence of HFOs correlates with seizure frequency, and introduction or withdrawal of antiepileptic drugs can affect both seizure occurrence and HFO rate.[64,65] Elucidation of the long-term dynamics of HFOs is a challenge that needs to be resolved, as well as clarification of whether HFOs can be used prospectively as a screening technique to predict epilepsy and whether they are a better marker of disease activity and epilepsy remission than the traditional hallmark, that is, interictal spikes. Further work is also necessary to establish the response of HFOs to antiepileptic drugs. In general, information about the response of HFOs to antiepileptic drugs is currently lacking, but ongoing studies are examining this clinically important topic. For instance, in the pilocarpine model, seizures and HFO rates decreased following the application of levetiracetam[65] or lacosamide.[66] The response to antiepileptic drugs has the potential to be used as a diagnostic test to differentiate between pathologic HFOs and physiologic ones.

HFOs in Models of Epilepsy

HFOs in the kainic acid and pilocarpine model of temporal lobe epilepsy

Much of our understanding of pathologic HFOs derives from studies carried out in the intrahippocampal kainic acid model of human temporal lobe epilepsy.[8,67] Most of these data have been summarized in previous reviews.[1,68] More recently, investigators used a unilateral suprahippocampal injection of kainic acid in mice that produced cell loss in the ipsilateral CA3, CA1, and hilar regions, as well granule cell dispersion and mossy fiber sprouting within 2 weeks after the injection.[69] The contralateral hippocampus, however,

was relatively unaffected. Overall the histopathologic changes seen in these experiments along with the appearance of seizures from the injected hippocampus reproduced several aspects of human TLE. Notably, silicon probes recorded pathologic HFOs (200–600 Hz) as early as 4 days after status epilepticus. During the development of epilepsy, pathologic HFOs in the CA1 and DG occurred in stable bursts, but the duration and spectral frequencies evolved from relatively brief bursts of low spectral frequency (~200 Hz) to longer duration bursts that contained higher frequencies between 400 and 450 Hz. The changes in duration and spectral power of pathologic HFOs could correspond with progressive neuronal disturbances associated with epileptogenesis.

The role of HFOs in ictogenesis and epileptogenesis has been further explored in the pilocarpine model of temporal lobe epilepsy. Pilocarpine-treated epileptic rats present with recurrent, spontaneous seizures that are characterized (even in the same animal) by LVF- or HYP-onset patterns.[42,70] As already stated in a previous section, these in vivo experiments have demonstrated that LVF-onset seizures have a prevalence of ripples preceding the seizure, whereas in HYP-onset seizures ripples predominate. Therefore, these findings suggest that different cellular or network mechanisms are responsible for initiating these two seizure-onset patterns in the pilocarpine model of temporal lobe epilepsy.

Development of epilepsy after traumatic brain injury

There are several models of traumatic brain injury that can be associated with varying degrees of focal or diffuse damage. Focal injuries typically include contusion, lacerations, and intracranial hematoma, whereas diffuse ones are usually in the form of widespread axonal, neuronal, or microvascular damage.[71] A well-characterized model of posttraumatic epilepsy, is the fluid percussion injury (FPI) rat model.[72] Most of the histologic studies in the FPI model were carried out within 2 months postinjury and have reported neurodegeneration, neurogenesis, astrocytosis, microgliosis, axonal and myelin injury, axonal sprouting, vascular damage, and angiogenesis within and surrounding the injured cortex and the underlying hippocampus and thalamus.

Electrophysiologic recordings obtained immediately after traumatic brain injury (i.e., <24 h postinjury) or later (<7 days), either in hippocampal slices maintained in vitro or in in vivo preparations, have shown hippocampal hyperexcitability and early seizures, whereas at >7 days after injury, similar recordings confirmed increased excitability and spontaneous seizures; these latter findings are the defining characteristics of posttraumatic epilepsy.[73,74] In FPI rats, a microelectrode and cortical screws recorded pathologic HFOs (100–600 Hz) in cortical areas adjacent to or within the injury core during the first 2 weeks following injury.[75] Moreover, pathologic HFOs have been reported to occur in almost 60% of FPI rats, but in none of the control

rats. In FPI rats that had completed long-term monitoring, pathologic HFOs were found in only those rats that later developed posttraumatic seizures, but were not observed in rats that did not develop late seizures.[75]

A new phenomenon was also observed by Bragin et al. in this study.[75] They found that a complex of repetitive HFOs superimposed on arcuate-shaped 10–16 Hz activity. These repetitive HFOs reflected population spikes with hypersynchronous multiunit firing, and were found only in those FPI rats that later developed seizures. There were clear differences between repetitive HFOs and sleep spindles, both of which were found in FPI rats, as well as in spike-and-wave discharges observed in some rodent strains. The appearance of repetitive HFOs could indicate disturbances in thalamocortical circuits, and their presence in cortical screw recordings only in FPI rats that develop late seizures make them a candidate for a noninvasive biomarker of post-traumatic epileptogenesis.

HFOs in the tetanus toxin model of temporal and neocortical epilepsy

The tetanus toxin model is a well-established model of temporal lobe epilepsy in which injection of a minute dose of tetanus toxin into the CA3 region of the hippocampus cause a chronic epileptic condition.[38,39,76] Approximately 1 week after tetanus toxin injection, the animals develop spontaneous and repeated seizures, which closely resemble complex partial seizures in humans, and can become secondarily generalized. In the tetanus toxin model, HFOs are observed between seizures, at their onset, and during their course. Although ripples are generated in both hippocampi, fast ripples occur predominantly or exclusively in the injected hippocampus.[38] Studies in the kainate model of epilepsy and in humans suggested that cell loss could be one of the key mechanisms involved in the pathogenesis of fast ripples.[14,22] However, experimental evidence obtained from the tetanus toxin model has shown that fast ripples can be generated even in the absence of obvious hippocampal sclerosis and major cell loss.[38]

Tetanus toxin injected into the neocortex is currently considered a model of neocortical epilepsy. When injected into the motor cortex, tetanus toxin manifests with partial motor status resembling epilepsia partialis continua, in which frequent interictal discharges are interspersed with seizures. It has been reported that in this model the neocortex also generates HFOs in the 70–160 Hz band, but no study has examined the presence of fast ripples in this model or explored the cellular mechanisms.[77]

HFOs and model of infantile spasms

Infantile spasms are a specific type of seizure in the developing brain associated with pathognomonic spasms, severe cognitive decline, intellectually disability, distinct EEG patterns known as hypsarrhythmia, and poor response to antiepileptic drugs. Chronic injection of the voltage-gated sodium channel antagonist, tetrodotoxin, during the early postnatal period (postnatal days 10–12), is one model of infantile spasms. Seizures in this experimental model physically mimic the spasms and produce hypsarrhythmia on EEG. HFOs with a frequency up to 600 Hz occur at the onset of seizures and during the interictal period in this experimental condition.[78,79] From the spatial perspective, HFOs were distributed over large areas of the neocortex of both hemispheres, but with amplitude predominance in the neocortex, contralateral to the tetrodotoxin injection site. An increasing intensity in HFOs was associated with an increased probability of transition to infantile spasms. These observations agree with clinical studies, which demonstrated the presence of HFOs during both spasms and interictal EEG in infants diagnosed with West syndrome.[80]

FUTURE DIRECTIONS OF HFO RESEARCH

This review shows the complexity of the "problem" that reflects how HFOs (and associated time-frequency features) are likely generated by multiple, possibly not exclusive, mechanisms at both cellular and network level. To fully elucidate the relationship between these observations and the presumptive underlying mechanisms, further studies are needed combining physiologically based models with experimental in vivo or in vitro techniques able to specifically target model-predicted key factors in small-scale networks. Future studies should also resolve unanswered questions about pathologic HFOs and bring direct evidence firmly demonstrating whether cellular and network mechanisms underlying pathologic HFOs play a causal role in ictogenesis and whether HFOs can induce permanent changes in brain structure and function that lead to the development of spontaneous seizures.

In recent years, we have observed dramatic advances in the development and application of optogenetic or chemogenetic techniques that allow neuron-specific and precise spatiotemporal control of neuronal activity, and optical labeling that allows monitoring thousands of cells simultaneously. Immediately after their discovery, it was predicted that these sophisticated techniques could determine the exact role of individual neuronal subtypes in HFOs, particularly in fast ripples. However, to date there have been only limited findings from these studies.

Much of the knowledge about the role of HFOs in animal models of epilepsy has been obtained from those presenting with a hippocampal origin, and less attention has been paid to extrahippocampal structures such as the neocortex. Whether similar pathophysiologic principles are involved in HFOs of neocortical origin needs to be clarified. This work, in combination with studies examining the causal role of HFOs in epileptogenesis, is important for establishing future directions of HFO research.

Identifying the various HFO-generating mechanisms may lead to assigning specific pathophysiologic interpretations to distinct HFO patterns detected in clinical recordings, accessible with currently available EEG-acquisition systems. This will enable expanding HFO classification beyond the traditional distinction between ripples and fast ripples, to include the fine details of the clinical, EEG, and pathophysiologic context. Although technical barriers to clinical use of HFOs still need to be addressed, a systematic approach to clinical HFO interpretation will help to establish their role as an adjunct to traditional EEG interpretation.

ACKNOWLEDGMENTS

DISCLOSURE OF CONFLICT OF INTERESTS

MA received a grant for preclinical studies from UCB PHARMA. The remaining authors have no conflicts of interest to disclose.

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