CRITICAL REVIEW AND INVITED COMMENTARY

How to record high-frequency oscillations in epilepsy: A practical guideline

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Summary

Objective: **Technology for localizing epileptogenic brain regions plays a central role in surgical planning. Recent improvements in acquisition and electrode technology have revealed that high-frequency oscillations (HFOs) within the 80–500 Hz frequency range provide the neurophysiologist with new information about the extent of the epileptogenic tissue in addition to ictal and interictal lower frequency events. Nevertheless, two decades after their discovery there remain questions about HFOs as biomarkers of epileptogenic brain and there use in clinical practice.**

Methods: **In this review, we provide practical, technical guidance for epileptologists and clinical researchers on recording, evaluation, and interpretation of ripples, fast ripples, and very high-frequency oscillations.**

Results: **We emphasize the importance of low noise recording to minimize artifacts. HFO analysis, either visual or with automatic detection methods, of high fidelity recordings can still be challenging because of various artifacts including muscle, movement, and filtering. Magnetoencephalography and intracranial electroencephalography (iEEG) recordings are subject to the same artifacts.**

Significance: **High-frequency oscillations are promising new biomarkers in epilepsy. This review provides interested researchers and clinicians with a review of current state of the art of recording and identification and potential challenges to clinical translation.**

KEY WORDS: **Ripples, High-frequency oscillations (HFOs), pHFOs, EEG, Magnetoencephalography.**

Recent advances in human brain electrophysiologic recordings have affected both clinical and basic neuroscience. Over the past three decades, the rise of broadband digital systems has enabled the monitoring of electrophysiologic signals beyond traditional low-pass filtered electroencephalography (EEG), extending the recordings to frequencies as high as 500 Hz and beyond. Based on these modern recording techniques, expanding evidence

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Key Points

- MEG and EEG can record ripples, and intracranial EEG can record ripples, fast ripples, and even very high-frequency oscillations
- Recording equipment can improve signal quality
- Muscle contractions, movement, and bad electrode contacts give rise to several artifacts that should be distinguished from epileptic HFOs
- Automatic detection methods are being developed and ready to be incorporated within EEG software

shows that high-frequency oscillations (HFOs) within the EEG signal, whether recorded invasively or with EEG or magnetoencephalography (MEG), contain information that can help us understand epileptogenicity and optimize the diagnosis and treatment of epilepsy.[1–3] Despite these recent advances in hardware and electrodes designed specifically to capture HFOs, these events are challenging to detect due to relative low signal-to-noise ratio compared to other interictal epileptiform discharges. They can occur as brief bursts lasting 30 msec or less. This review is meant to be useful to the physician as well as to advanced technologists and basic scientists. It is not meant to be comprehensive, but rather to address the most commonly used techniques in the field. This review sets forth routines of methodology, and provides guidance about recent developments of both recording electrodes and detection software. It is a collaborative effort from the 2nd international workshop on High Frequency Oscillations in Epilepsy held in Freiburg, Germany, on March 10–12, 2016

What Is an HFO?

Here we consider HFOs to include all physiologic and pathologic oscillatory activities within a limited frequency band from 80 to 500 Hz range that clearly stand out from the baseline and persist for at least four oscillation cycles. Depending on the HFO frequency range, these events are classified as ripples (80–250 Hz) and fast ripples (FR, >250 Hz). The time involving an HFO depends on the minimal frequency component that outlines the event, so a ripple event may last some tens of milliseconds, whereas a FR event may last a few milliseconds. It is important to note that definitions of HFOs vary in the literature, and the exact frequency content, filter settings, and manner of identification must be taken into consideration when interpreting the results.

How to Get Started?

To achieve the step from a research topic to an integral part of the clinical-decision process, several obstacles need to be overcome. First, one needs equipment for recording at a high sampling rate, which is at least three times the upper frequency of interest, to show HFOs below the cutoff frequency of the anti-aliasing filter and amplifiers with a low noise level for higher frequencies.[4] Because fast ripples are often small in amplitude, we recommend a noise amplitude level below ±2 μV for acquisition >250 Hz, which is achieved with some, commercially available amplifiers. For instance, from our personal experience we learned that, for example, Micromed LTM headboxes produce noise levels of around ±5 μV, whereas Micromed Flexi headboxes show lower noise. Special low-noise headboxes are being developed. Identification of ripples (80–250 Hz) benefits from a sampling rate >1,000 Hz, fast ripples (250–500 Hz) >2,000 Hz, and very fast HFOs (>1,000 Hz) above 5,000 Hz.[5] A sampling rate above the given values helps to distinguish events from artifacts, as artifacts often contain a broad spectrum of frequencies.[6]

Second, one needs software that allows a high-pass filter, preferentially a high-order finite impulse response (FIR) filter, of 80 Hz for ripples and 250 Hz for fast ripples. These filters are available within a few EEG software programs. If necessary, EEG recorded within one program can be reviewed as a European Data Format file (.EDF) in another program for the high frequencies or within an additional HFO analysis package (see below).

Third, one needs to learn to visually identify HFOs (Fig. 1). Learning how to identify HFOs is a process that does not differ from learning how to identify epileptiform discharges. It requires experience and knowledge of the (high-frequency) EEG signal to distinguish events from different types of artifacts (Table 1).[7] And similar to epileptiform spikes, HFO have rivaling artifacts and physiologic activity with overlapping spectral content. Marking HFOs might eventually be easier than marking spikes, as reflected by a higher interobserver agreement for HFOs. Several automatic detection programs are available (see below).[8] We strongly recommend use of these after comprehending the raw data.

The visual identification of HFOs is usually done after extending the amplitude toward 1 or 5 μV/mm and extending the time scale, for example, toward less than a second per page or to showing all samples recorded.[9] Sometimes it helps to have a limited number of channels on one screen. Marking can be done in any montage, but one should be aware that artifacts that occur in the reference channel, or in any one channel in an average montage, will contaminate signals and complicate the analysis. A bipolar montage is therefore often used. In research studies, events are often marked for a certain amount of time. Depending on the HFO rate (no. events/min), in some cases as little as 2 min allows the signal to yield stable results.[9] Marking all events separately is time-consuming. For clinical purpose, one can also review the signal and specify on which channels the fast ripples and ripples occur, that is, like the review of the EEG for

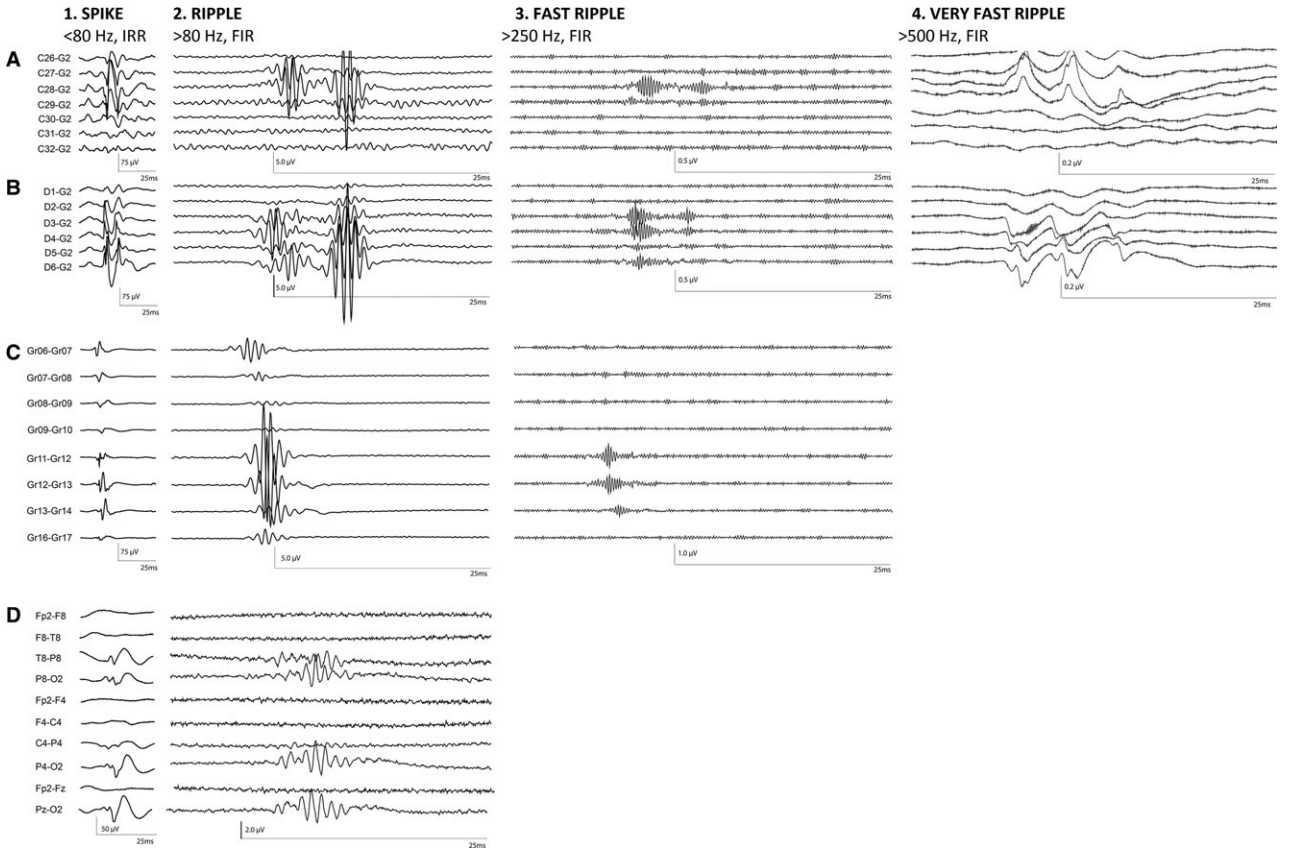

Figure 1.

Examples of HFOs recorded with different recording methods. (**A**) Long-term electrocorticography (grid) showing two spikes with simultaneous ripples and fast ripples. (**B**) Long-term depth recording, which was recorded simultaneously with the corticography (**A**). What can be seen are ripples and fast ripples that precede the cortical surface ripples and fast ripples slightly and very HFOs that can be recognized on one depth electrode channel. (**C**) Intraoperative electrocorticography showing a spike, a ripple, and a fast ripple. (**D**) Scalp EEG with bipolar double banana montage, showing only right-sided channels. A sharp wave and a ripple can be recognized.

spikes. Sometimes channels with nearly continuous HFO activity are seen, especially in the hippocampus.[10] Although associated with spikes and with distinct HFOs, this pattern was not clearly associated with the seizure-onset zone.

We advise starting with an EEG signal with limited artifacts, such as sleep EEG and invasive EEG recordings and with frequent epileptiform spikes. One can get used to recognizing epileptic HFOs by reviewing several interictal epileptiform spikes for the co-occurrence of HFOs. This can be done with a split window comparing normal EEG signal to high-pass-filtered EEG signal. Once you recognize HFOs co-occurring with spikes, you can get a feel for how they differ from filtering artifacts, muscle artifacts, electrode artifacts, or movement artifacts. An earlier review described and illustrated the typical morphology of these artifacts: wide frequency band activity for muscle artifacts, sharp peaks in the middle of the "HFO" for (single) electrode artifacts or 50 or 60 Hz activity, and large-amplitude artifacts over multiple electrodes in case of movement.[7] Then, one can try to find HFOs with similar waveform morphology. Reviewing invasive data is often done with a split screen showing 80 Hz filtered signal left and 250 Hz right. It is important to allow yourself time to build experience in visual identification of HFOs. It can be helpful to contact someone experienced in the field to verify findings.

False Identification of HFOs

Sharp events in the EEG can give rise to artifactual events that look similar to HFOs after filtering.[11] This holds for artifactual sharp events, but potentially also for epileptiform spikes, especially when infinite impulse response or finite impulse response filters having an impulse response with a significant number of oscillations are applied.[12] This can make the distinction between real HFOs on spikes and spike-related filter effects a challenge.[13] However, it is interesting to note that for clinical purposes, false and real spike-related HFOs may equally reflect epileptogenicity.[14] Another issue relevant to mention is that several studies

Table 1. Type of recording methods and the events that have been recognized with that method, typically encountered artifacts, and described physiologic activity per recording method

	Microelectrodes	Depth electrodes	Subdural grid electrodes	Intraoperative ECoG	EEG	MEG
Events recognized	Ripples Fast ripples Unit potentials	Ripples Fast ripples Very HFOs	Ripples Fast ripples Very HFOs	Ripples Fast ripples	Ripples Fast ripples?	Ripples
Artifacts	Electrode	Electrode Muscle (outer contacts) 50/60 Hz	Electrode Muscle 50/60 Hz	Movement Surrounding noise 50/60 Hz Electrode	Movement External pressure (O) Muscle (FT, T) Eye blinking (FT, F) 50/60 Hz ECG	External electromagnetic noise Movement Muscle Bad channels, 50/60 Hz, magnetocardiographic (MCG)
Physiological HF activity	Hippocampal ripples	Hippocampal ripples (inner contacts) Visual, sensorimotor (outer contacts)	Visual cortex Sensorimotor cortex	Sensorimotor cortex Visual cortex Broca's area?	Evoked sensorimotor responses	Evoked sensorimotor responses

HF, high frequency.

report on high-frequency activity as being HFOs. It is important to realize that the presence of high-frequency spectral content, for example, after Fourier transformation of the EEG signal, does not necessitate the presence of distinct HFOs.[11]

Recording Methods

Microelectrodes

The optimal spatial resolution for recording HFOs is a challenging question. Initially, ripple and FR oscillations were reported in humans when utilizing microelectrodes implanted in hippocampus (40 μm wires), and the FR were reported to be localized to ~mm^3 volumes.[11,15] Clinical intracranial electrodes, however, have a relatively large surface area (1–10 mm^2) and are separated by 5–10 mm (center-to-center). Spatial spectral analysis of high-density electrocorticography (ECoG) suggests the optimal spacing of electrodes for capturing the spatial variation of gamma frequency (25–100 Hz) local field potential (LFP) amplitude modulation to be ~1.25 mm in human cortex.[16] Although these studies suggest at least ~mm scale spatial resolution may be required for recording physiologic LFP oscillations in human brain, the optimal scale for pathologic oscillations and seizures remains unclear. The assumption that pathologic LFP defining interictal abnormalities and seizures are well-behaved functions and can be constructed by superposition of microscale recordings may also not hold.[17]

There remain significant technical challenges for probing the brain over wide spatiotemporal scales required for pursuing fundamental questions about the origin of seizures and HFOs. For example, if the goal is to explore the role of individual neurons in generation of focal seizures, the electrophysiologic recordings must span neurons to clinical scale resolutions (~10–10,000 μm). This presents a major technical challenge, since 100 μm resolution over 1 cm^2 would require 10,000 channel recordings. High-resolution neuronal and LFP recordings show promise for basic research[18–21] and improved understanding of seizure generation.[22–24]

Depth electrodes

The clinical depth electrodes represent perhaps the optimal method of recording HFOs. They have been shown to provide robust HFO recordings,[13,25,26] representing a good compromise between recording at microscale recordings that show HFO organization at a sub-millimeter scale[26–29] and macroscale.[30] Clinical depth electrodes have a good spatial selectivity and can reach nearly any target in the brain, including mesiotemporal structures, which are the most investigated areas generating HFOs. Being invasive, they show fewer muscle and eye movement artifacts. Despite their invasiveness, they have an excellent surgical safety record,[31] and a common trend in minimizing invasiveness is to decrease their diameter (0.8 mm; DIXI Medical, Besancon, France) and contact length (1.32 mm; AdTech, Racine, WI, U.S.A.) to decrease the area from which the intracranial EEG (iEEG) signals are being recorded below 5 mm^2. At this level of the contact surface, the electrode–tissue interface still has an impedance low enough to allow the use of standard EEG cables and recording equipment (Fig. 2).

Grid electrodes

Subdural grid electrodes are implanted in presurgical epilepsy diagnosis for up to 2 weeks and need medical device approval from legal authorities, that is, Conformité Européenne (CE) mark in Europe or U.S. Food and Drug Administration (FDA) approval in the United States. Standard grid

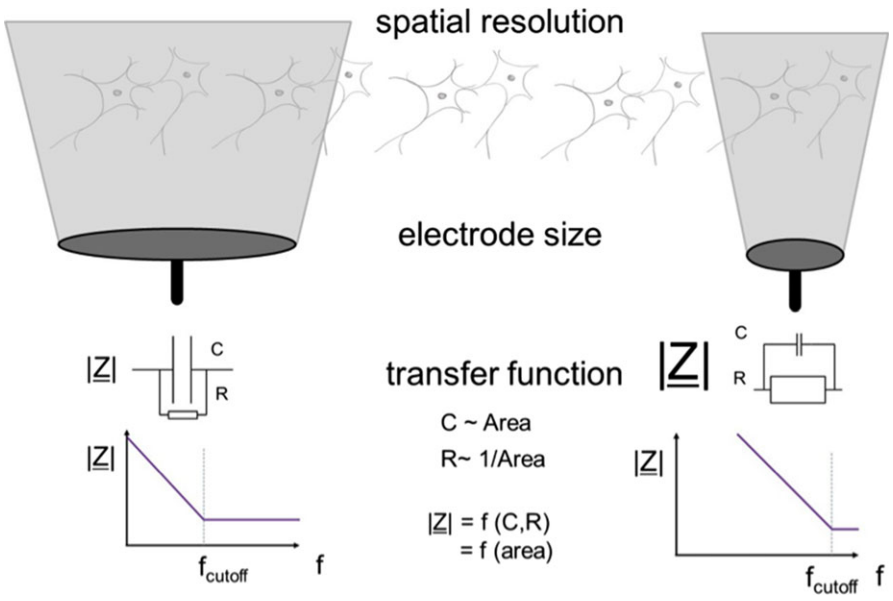

Figure 2.
Influence of the size of recording electrodes on spatial resolution and recording properties (transfer function). Small electrodes allow for recording of small nerve cell ensembles (right) but result in high impedance magnitude (|Z|). Large electrode sites (left) exhibit large capacitive and small resistive values, which results in lower impedance at low frequencies and a low cutoff frequency from which signal attenuation is constant.

and strip electrodes approved for clinical use meet the requirements to record HFOs with respect to spatial resolution and their electrical properties. The same methodologic discussions hold for depth electrodes, but from the degrees of freedom of manufacturing grid electrodes and from applications beyond epilepsy, like for brain–computer interface, the issues are more varied for grid electrodes. The smaller the size of an electrode the better the spatial resolution, and ensembles of neurons can be recorded. However, the smaller the electrode size the smaller the interface capacity, and the transfer resistance is larger (Fig. 2).[32] A smaller electrode size and thus larger transfer resistance and impedance is associated with a higher cutoff frequency above which the impedance magnitude stays constant and the desired region for recording of signals (Fig. 2). To solve the dilemma of high spatial selectivity and low impedance and cutoff, frequency grid arrays with high spatial resolution have been developed. Figure 3 shows the clinically approved subdural electrode grid arrays next to current technologic solutions targeting higher density and increased flexibility.[18,20,21,33] Subdural grid electrodes are more likely than depth electrodes to pick up noise and artifacts.

Intraoperative electrocorticography

Tailoring the resection of the epileptogenic tissue with intraoperative recording of spikes with electrocorticography is a method that has been disputed. It works well for dysplasias showing rhythmic spiking patterns.[34,35] ECoG can be recorded with grid electrodes and subdural strips. Subdural grids can be combined with depth electrodes directed at the lesion.[36] HFOs can be distinguished intraoperatively if anesthetics like propofol are temporarily stopped.[37] Fast ripples seem to be good predictors of outcome, especially if they remain after the resection.[38–40] Recording and interpreting fast ripples during surgery is feasible, and a first trial testing of the use of HFOs during epilepsy surgery has started.[41] In this setup, propofol is ceased and ECoG is recorded for at least 5 min per recording. The operation theater introduces many artifacts, and it helps to switch off all neighboring electronics and stabilize the grids with cotton compress. Data can be visually reviewed off-line, which takes about 5 min per recording of 5 min. The speed of review could be increased with the use of (online) automatic HFO detectors (see below), other signal analysis methods, or by evoking high frequency responses with single pulse stimulation.[42–44]

Scalp EEG

Some EEG textbooks taught us that the skull filters away higher frequencies and it was not expected to find epileptic HFOs with scalp EEG. This is not correct, as the skull does not have low-pass filter capacities. It diminishes signal amplitude related to distance between generators and sensors, but so long as the signal amplitude is greater than noise, the high-frequency signal can be distinguished. With an increase in frequency, the signal power falls off by at least f^{-1} (one over the frequency), but the noise-level diminishes concordantly.[45] Another argument is that a cortical patch of minimal 7 cm^2 with synchronous activity is required to enable picking up of the signal outside the skull.[46] This argument does not necessarily hold, and there are likely cases for which the superposition of nonspatially contiguous generators can generate detectable signals. In general, the scalp EEG can record HFOs, so long as the EEG samples at the right spot and the signal has sufficient signal-to-noise level. This was shown for clinical recordings in focal epilepsy, West syndrome, Rolandic spikes, and possibly generalized epilepsy.[30,47–49] A comparative study with

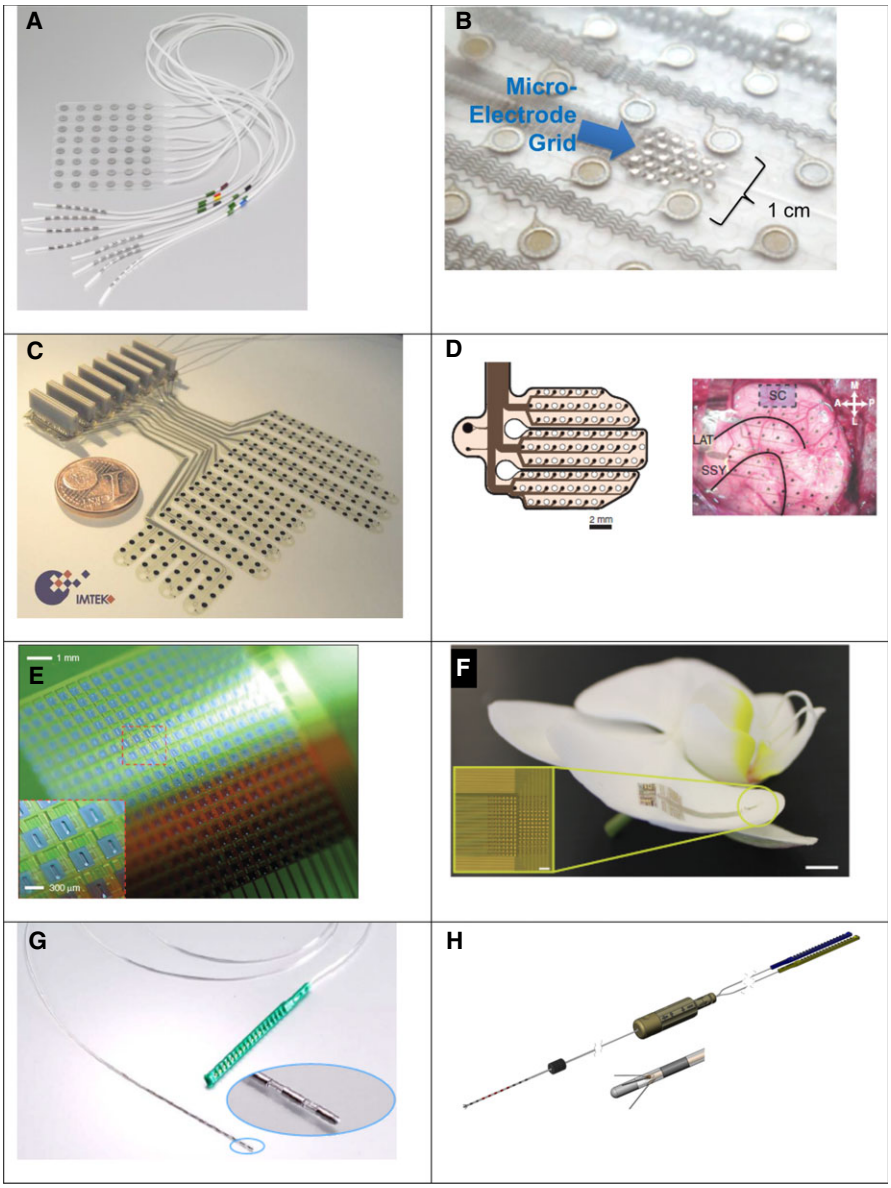

Figure 3.
Examples of grid and depth electrodes. Grid arrays (**A**–**F**) and depth electrodes (**G**–**H**). (**A**) clinical standard subdural grid (DIXI Medical) (**B**) micro-macro hybrid grid, under medical device approval for human applications (CorTec GmbH, Germany), (**C**, **D**) micromachined polyimide substrates for higher spatial selectivity, (**E**) integrated switching electronics, (**F**) amplification at the electrode site with polymer-based transistors, (**G**) clinical standard depth electrode, and (**H**) hybrid micro–macro electrodes (DIXI Medical). Main advances in the development of grids focus on higher spatial sampling, which requires new solutions for wiring and amplification. *Epilepsia* © ILAE

EEG and simultaneous subdural electrodes showed that HFOs on scalp co-occurred with HFOs on ECoG.[50] Recording of HFOs with regular clinical EEG enables better diagnosis preoperative noninvasive localization of the HFO area, and might help to monitor therapeutic effects. High-density EEG may yield better identification of HFOs than the regular EEG configuration, as the HFO area is usually small and can thus be undersampled. In addition,, high-density EEG measurements, likewise in MEG, can allow spatial filtering of artifacts. This might also be overcome with special concentric-ring electrodes.[51]

Magnetoencephalography

There is increasing evidence that HFOs can be noninvasively detected using MEG. HFOs within the epileptic focus can be visually detected in the ripple band in MEG traces, whereas HFOs outside the epileptic focus are rarely detected. The rate of visually detected HFOs in MEG is usually low (<0.5/min), and they often, but not exclusively, co-occur with epileptic spikes.[52–54]

One of the advantages of MEG is that it covers the head with high sensor density. This means that it is challenging to visually evaluate all physical MEG channels, as it is often done in iEEG. Thus semi-automatic approaches with automatic pre-detection and subsequent visual evaluation have been applied and were found to be useful.[54] The visual detection rate of HFOs in MEG is influenced by the distance between sensors and HFO generators as well as by external noise such as instrumentation noise. Virtual sensors can facilitate the detection of HFOs by reducing noise.[53] As in scalp EEG, artifacts in MEG can be visually distinguished from HFOs.[54]

Beyond identifying HFOs in MEG traces, their generators can be localized within brain structures. Advanced frequency domain source-imaging techniques, such as the

wavelet Maximum Entropy on the Mean (wMEM) and beamformer methods, can be applied to enable quantitative comparisons with iEEG and might ultimately facilitate non-invasive localization of the HFO area.[54–57]

Available Software for Automatic Detections

High absolute numbers of HFOs, the need for trained experts, and the time-consuming process of visual HFO evaluation has led to the development of automatic detection and analysis packages.

Detectors can be grouped based on the first processing stage, either filtering the EEG to the HFO frequency band or time frequency analysis (Fig. 4).[8,58–61] Typical parameters derived from the filtered signals track the higher amplitude during the oscillation compared to the background. A better characterization of the baseline properties and a subsequent adaptation of the classification can be obtained using the entropy of time–frequency transformation results and may also contribute to their impact with respect to epileptogenicity.[9,14,62] The oscillatory nature of the pattern is either ensured by counting of the extrema,[58] instantaneous frequency estimated by the Hilbert transformation, or narrowband filtering.[63,64] Artificial neural networks or machine learning methods may be applied in the classification stage.[63] Final output parameters can be the localization, time, and duration of HFOs, but can also aim for a more fine-grained characterization.[14,64–66]

The application of a high-pass filter, either with FIR or infinite impulse response (IIR) design as a first processing step, comes with two disadvantages. First, the application of the filter results in oscillatory patterns not only for oscillations buried in the low-frequency background but also for all kinds of sharp transients (spikes, artifacts).[12] Second, important information from lower frequencies is no longer available, which may be needed for the distinction between spike-associated HFOs and distinct HFOs.[14,67] Time–frequency transformations form a starting point for feature extraction (power or power ratios, separation of high-frequency activity from lower frequency activity by a trough, identification of blobs, or computer vision).[68–70] Basic principles of classification are similar to that for the classification of features derived from the filtered signal but with additional output options, which allow one to distinguish between pure and spike-associated HFOs.

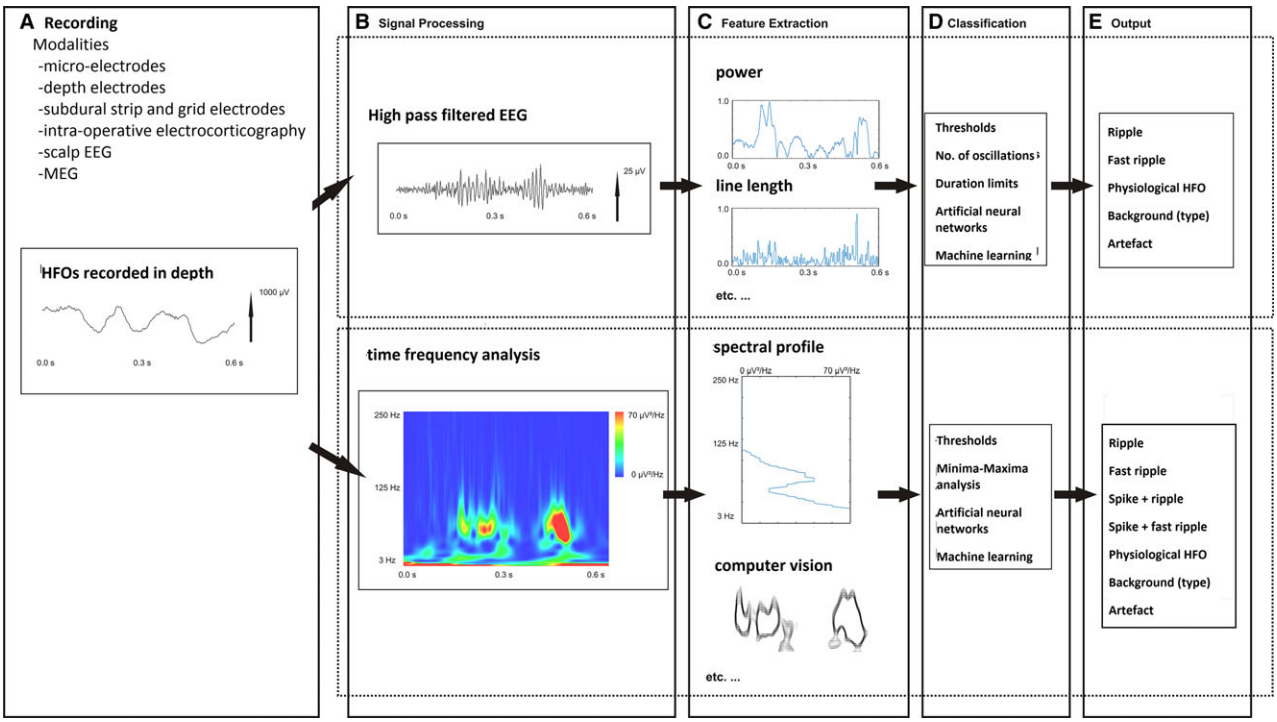

Figure 4.

The essence of different automated HFO detectors: (**A**) Broadband electrophysiologic recording of brain activity. (**B**) First detector stage consisting either of filtering the recordings to the HFO frequency bands or performing time–frequency analysis on the broadband recordings. (**C**) Feature extraction, for example, based on higher signal amplitudes during the oscillation in the filtered recordings, spectral profiles, or computer vision approaches applied to time–frequency analysis. (**D**) Wide range of classification approaches: From simple thresholding to modern machine learning approaches. (**E**) Results of the detector output are extended from ripple and fast ripple distinction to subclassifications, for example, differentiating between independent and spike-coupled HFOs.

Most HFO detection approaches are developed as research tools and may be made available by the research groups on request and require individual agreements (Department of Neurology, Paracelsus Medical University, Salzburg, Austria).[68,69] RIPPLELAB is a MATLAB toolbox that is available to the research community (https://github.com/BSP-Uniandes/RIPPLELAB) under terms of the GNU General Public License.[71] It contains a wide range of tools for visual and automatic HFO annotation and validation. The standard EEG software programs are starting to offer HFO-reviewing toolboxes in which automatic detectors may eventually be integrated. When buying such software, it is essential to verify that the right filter settings have been used and that the review enables easy comparison of the EEG in regular settings and HFO settings.

Recent advances in computer technologies have made the analysis of HFO less time-consuming. Nevertheless, the automatic detection of HFOs is challenging and it is not possible to develop "black box"-like techniques that are a panacea to all detection problems. There is, however, no doubt that the analysis of neuronal oscillations is still open to considerable progress. All current methods face the same difficulties: For example, the presence of noise and multiple oscillations might destroy the discernibility of spectral peaks representing rhythmic activity. Finally, calibration was often performed in a semi-supervised way, whereby human experts review sampled candidate events identified by the automated detector. Often, the methods are optimized for a particular database and the detection is limited by investigator experience, inducing possible bias, low inter-rater reliability, and problems with reproducibility.[72] Therefore, automatic detection results should be integrated into clinical review software. This should include an adapted display of the detection results to allow visual checking of detections, as a gold standard for automatic detection is still missing. The cooperation between research centers, adopting common analysis procedures combined with the sharing of wide bandwidth data, will help to standardize automated detection strategies of HFOs.

HFOs and Other Brain Rhythms

The research community is increasingly interested in the co-occurrence of ripples and fast ripples with other brain rhythms. A specific reason for this interest is that it might help us understand and distinguish physiologic and epileptic HFOs.

Infraslow activity and HFOs

Frequencies on the very low limit of the frequency spectrum, like ictal, very slow, and infraslow (DC) activity, are thought to contain important information and might be desirable to record together with the high-frequency signals. A DC amplifier or an AC amplifier with long time constant, that is, 10 s, can be used to record slow shifts as long as the following three conditions are carefully considered from the methodologic point of view: (1) the kind of metals used for electrodes, (2) the size of the recording surface, and (3) the input impedance of the amplifier.[73] With regard to (1), nonpolarizable or reversible electrodes are essential to minimize DC electrode potentials that could distort slow potential signals. Currently available nonpolarizable electrodes are made of Ag/AgCl. However, nonpolarizable metals including Ag/AgCl are toxic to the brain tissue. Therefore, for intracranial electrodes, the only polarizable metals are available, such as platinum, stainless steel, and gold.[74] As for (2) and (3), impedance (i.e., resistance and capacitance) of the electrodes combined with the input impedance of the amplifier must be considered to obtain optimal recordings. Because the capacitance is proportional to the electrode surface area (Fig. 2), electrodes of large size such as subdural contacts only minimally attenuate slow potentials, since the cutoff frequency of these electrodes is low. Currently, commercially available EEG systems usually have input amplifiers with a sufficiently high impedance, >50 MOhm, that even clinical depth electrodes are recorded without signal distortion. To minimize imbalance of electrode potentials, use of a system reference made of platinum is recommended, even if employed as a surface or scalp electrode such as a mastoid electrode.

Very high-frequency oscillations

Very high-frequency oscillations (VHFOs), faster than 1,000 Hz, have been detected in localized areas by subdural macroelectrodes in patients with neocortical epilepsy and may be more specific to the core of the epileptogenic zone than HFOs.[5,75] To visualize VHFOs, the EEG was recorded with a 10-kHz sampling rate, the horizontal (time) and vertical (amplitude) axes of the EEG display expanded, with the EEG digitally high-pass filtered at 160 Hz and low-pass filtered at 3 kHz. Peaks of VHFOs were visually identified on a computer screen, and the frequency, amplitude, and duration were measured. Because the amplitude of VHFOs are very low and duration is very short, magnification of the horizontal (time) and vertical (amplitude) axes is essential for visual detection. To exclude the possibility of a "false" high-frequency component produced by filter, EEG data high-pass filtered at 160 Hz and those at 53 Hz are compared. VHFOs were considered true if the frequencies of the oscillations at both filter settings were the same. Waveforms of VHFOs are not typical sinusoidal waves, and fluctuations in frequency and amplitude for individual peaks appear in waveforms. Interictally, VHFOs appear intermittently, followed by spikes. Therefore, a simple approach to detect VHFOs is marking spikes in conventional EEG settings and searching high-frequency activities around the marked spikes.

Cross-coupling with other rhythms

Combined recording of both HFOs and other rhythms, especially of DC shifts and delta rhythms, depends on how

bandpass filter and sampling rate are set for appropriate wideband EEG recording. Coupling with other rhythms could be applicable for both ictal and interictal activities. Ictal activity was described earlier. For interictal activity, as opposed to ictal DC shifts of long duration, rather short, fragmented slow activity of delta frequency or slower activity has been observed. Co-occurrence of HFOs and slow activity of delta frequency or slower might help to delineate so-called "red slow or epileptic slow," instead of red-spike. Especially slow waves of sleep show coupling with presumed epileptic HFOs on the slope of the slow wave and with presumed physiologic HFOs after the peak of the slow wave.[65] Other studies showed that cross-frequency amplitude-to-phase coupling between HFOs and the phase of theta and alpha rhythms, more than delta, was found to be elevated in the seizure-onset zone.[76,77] It is difficult to detect co-occurrence of HFOs and slow activity of delta only by visual inspection of wave form simultaneously, and thus in addition to the actual recording condition, appropriate analysis methods are needed as the next step. An example is the modulation index, which reflects the strength of coupling between amplitude of HFOs and the phase of slow waves.[78]

Conclusion and Future Perspectives

HFOs are novel electromagnetic biomarkers for epileptogenic brain tissue, and we are standing at the threshold of a change in clinical practice and basic understanding of epilepsy. Several steps need to be taken for the clinical community to overcome the hindrances impeding the implementation of HFOs in clinical practice, which includes knowledge and technical capabilities and general in-depth understanding of the pathophysiology and further development of signal recording and analysis methods.

The 2nd international workshop on High Frequency Oscillations in Epilepsy held in Freiburg, Germany, included a teaching workshop. This kind of teaching needs to be repeated and extended to increase comprehension. Meanwhile, software producers are currently extending the capabilities by building in HFO filters and analysis methods. Despite the existence of a couple of publications describing and evaluating automatic detection algorithms, only a few publications focus on the application of the detector. Their application should be encouraged despite their limitations as complements to visual HFO assessment and to open new opportunities, where visual HFO analysis is not feasible due to its extreme time demands, like in multichannel recordings. Electrode sensor diversity is increasing and preclinical research demands for higher channel counts and smaller dimensions continue to drive development. Recording devices can be developed with a focus on low noise and artifacts. Translation of these preclinical devices into medical products, however, is an expensive and long-lasting endeavor since legal requirements on medical device approval are continuously rising. The number of patients is still relatively low, so the economic drive is low for companies to go beyond small evolutionary development steps. Public incentives are needed to initiate public–private partnerships to help disruptive technologies come to the market for new therapies.

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Disclosure of Conflicts of Interest

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References

1. Le Van Quyen M. This special issue of Progress in Neurobiology is dedicated to the meeting held at the Montreal Neurological Institute, Canada. Introduction. *Prog Neurobiol* 2012;98:239–240.
2. Jiruska P, Alvarado-Rojas C, Schevon CA, et al. Update on the mechanisms and roles of high-frequency oscillations in seizures and epileptic disorders. *Epilepsia* 2017; https://doi.org/10.1111/epi.13830.
3. Frauscher B, Bartolomei F, Kobayashi K, et al. High-frequency oscillations (HFOs) in clinical epilepsy care. *Epilepsia* 2017; https://doi.org/10.1111/epi.13829.
4. Scheer HJ, Sander T, Trahms L. The influence of amplifier, interface and biological noise on signal quality in high-resolution EEG recordings. *Physiol Meas* 2006;27:109–117.
5. Usui N, Terada K, Baba K, et al. Very high frequency oscillations (over 1000 Hz) in human epilepsy. *Clin Neurophysiol* 2010;121:1825–1831.
6. Otsubo H, Ochi A, Imai K, et al. High-frequency oscillations of ictal muscle activity and epileptogenic discharges on intracranial EEG in a temporal lobe epilepsy patient. *Clin Neurophysiol* 2008;119:862–868.
7. Zijlmans M, Jiruska P, Zelmann R, et al. High-frequency oscillations as a new biomarker in epilepsy. *Ann Neurol* 2012;71:169–178.
8. Zelmann R, Mari F, Jacobs J. A comparison between detectors of high frequency oscillations. *Clin Neurophysiol* 2012;123:106–116.
9. Zelmann R, Zijlmans M, Jacobs J, et al. Improving the identification of high frequency oscillations. *Clin Neurophysiol* 2009;120:1457–1464.
10. Mari F, Zelmann R, Andrade-Valenca L, et al. Continuous high-frequency activity in mesial temporal lobe structures. *Epilepsia* 2012;53:797–806.

11. Worrell GA, Jerbi K, Kobayashi K, et al. Recording and analysis techniques for high-frequency oscillations. *Prog Neurobiol* 2012;98:265–278.
12. Bénar CG, Chauvière L, Bartolomei F, et al. Pitfalls of high-pass filtering for detecting epileptic oscillations: a technical note on 'false' ripples. *Clin Neurophysiol* 2010;121:301–310.
13. Amiri M, Lina JM, Pizzo F, et al. High frequency oscillations and spikes: separating real HFOs from false oscillations. *Clin Neurophysiol* 2016;127:187–196.
14. Burnos S, Frauscher B, Zelmann R, et al. The morphology of high frequency oscillations (HFO) does not improve delineating the epileptogenic zone. *Clin Neurophysiol* 2016;127:2140–2148.
15. Bragin A, Wilson CL, Staba RJ, et al. Interictal high-frequency oscillations (80–500 Hz) in the human epileptic brain: entorhinal cortex. *Ann Neurol* 2002;52:407–415.
16. Freeman WJ, Rogers LJ, Holmes MD, et al. Spatial spectral analysis of human electrocorticograms including the alpha and gamma bands. *J Neurosci Methods* 2000;95:111–121.
17. Mandelbrot B. *Gaussian self-affinity and fractals: globality, the earth, 1/f noise, and R/S*. New York: Springer-Verlag, 2002.
18. Viventi J, Kim DH, Vigeland L, et al. Flexible, foldable, actively multiplexed, high-density electrode array for mapping brain activity in vivo. *Nat Neurosci* 2011;14:1599–1605.
19. Alvarado-Rojas C, Huberfeld G, Baulac M, et al. Different mechanisms of ripple-like oscillations in the human epileptic subiculum. *Ann Neurol* 2015;77:281–290.
20. Khodagholy D, Gelinas JN, Thesen T, et al. NeuroGrid: recording action potentials from the surface of the brain. *Nat Neurosci* 2015;18:310–315.
21. Rubehn B, Bosman C, Oostenveld R, et al. A MEMS-based flexible multichannel ECoG-electrode array. *J Neural Eng* 2009;6:036003.
22. Stead M, Bower M, Brinkmann BH, et al. Microseizures and the spatiotemporal scales of human partial epilepsy. *Brain* 2010;133:2789–2797.
23. Schevon CA, Weiss SA, McKhann G, et al. Evidence of an inhibitory restraint of seizure activity in humans. *Nat Commun* 2012;3:1060.
24. Truccolo W, Donoghue JA, Hochberg LR, et al. Single-neuron dynamics in human focal epilepsy. *Nat Neurosci* 2011;14:635–641.
25. Urrestarazu E, Chandler R, Dubeau F, et al. Interictal high-frequency oscillations (100–500 Hz) and the intracerebral EEG of epileptic patients. *Brain* 2007;130:2354–2366.
26. Worrell GA, Gardner AB, Stead SM, et al. High-frequency oscillations in human temporal lobe: simultaneous microwire and clinical macroelectrode recordings. *Brain* 2008;131:928–937.
27. Bragin A, Engel JJ, Wilson CL, et al. High-frequency oscillations in human brain. *Hippocampus* 1999;9:137–142.
28. Schevon CA, Trevelyan AJ, Schroeder CE, et al. Spatial characterization of interictal high frequency oscillations in epileptic neocortex. *Brain* 2009;132:3047–3059.
29. Staba RJ, Wilson CL, Bragin A, et al. High-frequency oscillations recorded in human medial temporal lobe during sleep. *Ann Neurol* 2004;56:108–115.
30. Andrade-Valenca LP, Dubeau F, Mari F, et al. Interictal scalp fast oscillations as a marker of the seizure onset zone. *Neurology* 2011;77:524–531.
31. Cardinale F, Cossu M, Castana L, et al. Stereoelectroencephalography: surgical methodology, safety, and stereotactic application accuracy in 500 procedures. *Neurosurgery* 2013;72:353–366.
32. Stieglitz T. Electrodes. In Brette R, Destexhe A (Eds) *Handbook on neural activity measurement*. Oxford: Oxford University Press, 2012:8–43.
33. Stitt I, Galindo-Leon E, Pieper F, et al. Intrinsic coupling modes reveal the functional architecture of cortico-tectal networks. *Sci Adv* 2015;1:e1500229.
34. Ferrier CH, Aronica E, Leijten FS, et al. Electrocorticographic discharge patterns in glioneuronal tumors and focal cortical dysplasia. *Epilepsia* 2006;47:1477–1486.
35. Morales Chacon L, Estupinán B, Lorigados Pedre L, et al. Microscopic mild focal cortical dysplasia in temporal lobe dual pathology: an electrocorticography study. *Seizure* 2009;18:593–600.
36. Privitera MD, Quinlan JG, Yeh HS. Interictal spike detection comparing subdural and depth electrodes during electrocorticography. *Electroencephalogr Clin Neurophysiol* 1990;76:379–387.
37. Zijlmans M, Huiskamp GM, Cremer OL, et al. Epileptic high-frequency oscillations in intraoperative electrocorticography: the effect of propofol. *Epilepsia* 2012;53:1799–1809.
38. Wu JY, Sankar R, Lerner JT, et al. Removing interictal fast ripples on electrocorticography linked with seizure freedom in children. *Neurology* 2010;75:1686–1694.
39. van Klink NE, van't Klooster MA, Zelmann R, et al. High frequency oscillations in intraoperative electrocorticography before and after epilepsy surgery. *Clin Neurophysiol* 2014;125:2212–2219.
40. van't Klooster MA, van Klink NE, Leijten FS, et al. Residual fast ripples in the intraoperative corticogram predict epilepsy surgery outcome. *Neurology* 2015;85:120–128.
41. van't Klooster MA, Leijten FS, Huiskamp G, et al. High frequency oscillations in the intraoperative ECoG to guide epilepsy surgery ("The HFO Trial"): study protocol for a randomized controlled trial. *Trials* 2015;16:422.
42. Zweiphenning WJ, van't Klooster MA, van Diessen E, et al. High frequency oscillations and high frequency functional network characteristics in the intraoperative electrocorticogram in epilepsy. *Neuroimage Clin* 2016;12:928–939.
43. Geertsema EE, van't Klooster MA, van Klink NE, et al. Non-harmonicity in high-frequency components of the intra-operative corticogram to delineate epileptogenic tissue during surgery. *Clin Neurophysiol* 2016;128:153–164.
44. van't Klooster MA, Zijlmans M, Leijten FS, et al. Time-frequency analysis of single pulse electrical stimulation to assist delineation of epileptogenic cortex. *Brain* 2011;134:2855–2866.
45. von Ellenrieder N, Beltrachini L, Perucca P, et al. Size of cortical generators of epileptic interictal events and visibility on scalp EEG. *NeuroImage* 2014;94:47–54.
46. Tao JX, Ray A, Hawes-Ebersole S, et al. Intracranial EEG substrates of scalp EEG interictal spikes. *Epilepsia* 2005;46:669–676.
47. Kobayashi K, Akiyama T, Oka M, et al. A storm of fast (40–150 Hz) oscillations during hypsarrhythmia in West syndrome. *Ann Neurol* 2015;77:58–67.
48. van Klink NE, van't Klooster MA, Leijten FS, et al. Ripples on rolandic spikes: a marker of epilepsy severity. *Epilepsia* 2016;57:1179–1189.
49. Pizzo F, Ferrari-Marinho T, Amiri M, et al. When spikes are symmetric, ripples are not: bilateral spike and wave above 80 Hz in focal and generalized epilepsy. *Clin Neurophysiol* 2016;127:1794–1802.
50. Zelmann R, Lina JM, Schulze-Bonhage A, et al. Scalp EEG is not a blur: it can see high frequency oscillations although their generators are small. *Brain Topogr* 2014;27:683–704.
51. Besio WG, Martínez-Juárez IE, Makeyev O, et al. High-frequency oscillations recorded on the scalp of patients with epilepsy using tripolar concentric ring electrodes. *IEEE J Transl Eng Health Med* 2014;2:2000111.
52. Heers M, Jacobs J, Hirschmann J, et al. Spike associated high frequency oscillations in magnetoencephalography co-localize with focal cortical dysplasia type IIB. *Epilepsia* 2013;54:23–24.
53. van Klink N, Hillebrand A, Zijlmans M. Identification of epileptic high frequency oscillations in the time domain by using MEG beamformer-based virtual sensors. *Clin Neurophysiol* 2016;127:197–208.
54. von Ellenrieder N, Pellegrino G, Hedrich T, et al. Detection and magnetic source imaging of fast oscillations (40–160 Hz) recorded with magnetoencephalography in focal epilepsy patients. *Brain Topogr* 2016;29:218–231.
55. Gross J, Kujala J, Hämäläinen MS, et al. Dynamic imaging of coherent sources: studying neural interactions in the human brain. *Proc Natl Acad Sci USA* 2001;98:694–699.
56. Lina JM, Chowdhury R, Lemay E, et al. Wavelet-based localization of oscillatory sources from magnetoencephalography data. *IEEE Trans Biomed Eng* 2014;61:2350–2364.
57. Grova C, Aiguabella M, Zelmann R, et al. Intracranial EEG potentials estimated from MEG sources: a new approach to correlate MEG and iEEG data in epilepsy. *Hum Brain Mapp* 2016;37:1661–1683.
58. Staba RJ, Wilson CL, Bragin A, et al. Quantitative analysis of high-frequency oscillations (80–500 Hz) recorded in human epileptic hippocampus and entorhinal cortex. *J Neurophysiol* 2002;88:1743–1752.
59. Gardner AB, Worrell GA, Marsch E. Human and automated detection of high-frequency oscillations in clinical intracranial EEG recordings. *Clin Neurophysiol* 2007;118:1134–1143.

60. Crépon B, Navarro V, Hasboun D, et al. Mapping interictal oscillations greater than 200 Hz recorded with intracranial macroelectrodes in human epilepsy. *Brain* 2010;133:33–45.
61. Dümpelmann M, Jacobs J, Kerber K, et al. Automatic 80–250 Hz 'ripple' high frequency oscillation detection in invasive subdural grid and strip recordings in epilepsy by a radial basis function neural network. *Clin Neurophysiol* 2012;123:1721–1731.
62. Kerber K, Dümpelmann M, Schelter B, et al. Differentiation of specific ripple patterns helps to identify epileptogenic areas for surgical procedures. *Clin Neurophysiol* 2014;125:1339–1345.
63. Dümpelmann M, Jacobs J, Schulze-Bonhage A. Temporal and spatial characteristics of high frequency oscillations as a new biomarker in epilepsy. *Epilepsia* 2015;56:197–206.
64. von Ellenrieder N, Andrade-Valença LP, Dubeau F, et al. Automatic detection of fast oscillations (40–200 Hz) in scalp EEG recordings. *Clin Neurophysiol* 2012;123:670–680.
65. von Ellenrieder N, Frauscher B, Dubeau F, et al. Interaction with slow waves during sleep improves discrimination of physiologic and pathologic high-frequency oscillations (80–500 Hz). *Epilepsia* 2016;57:869–878.
66. Lachner Piza D, Bruder JC, Jacobs J, et al. Differentiation of spindle associated hippocampal HFOs based on a correlation analysis. 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC'16).
67. Jacobs J, Kobayashi K, Gotman J. High-frequency changes during interictal spikes detected by time-frequency analysis. *Clin Neurophysiol* 2011;122:32–42.
68. Burnos S, Hilfiker P, Sürücü O, et al. Human intracranial high frequency oscillations (HFOs) detected by automatic time-frequency analysis. *PLoS ONE* 2014;9:e94381.
69. Donos C, Mindruta I, Maliia MD, et al. A computer vision approach to automatic detection of high frequency oscillations. 2nd International Workshop on High Frequency Oscillations in Epilepsy, March 10–12 Freiburg Germany.
70. Kobayashi K, Merlet I, Gotman J. Separation of spikes from background by independent component analysis with dipole modelling and comparison to intracranial recording. *Clin Neurophysiol* 2001;112:405–413.
71. Navarrete M, Alvarado-Rojas C, Le Van Quyen M, et al. RIPPLELAB: a comprehensive application for the detection, analysis and classification of high frequency oscillations in electroencephalographic signals. *PLoS ONE* 2016;11:e0158276.
72. Menendez de la Prida L, Staba RJ, Dian JA. Conundrums of high-frequency oscillations (80–800 Hz) in the epileptic brain. *Clin Neurophysiol* 2015;32:207–219.
73. Ikeda A. DC recordings to localize the ictal onset zone. In Lüders HO (Ed) *Textbook of epilepsy surgery*. London: Informa UK Ltd, 2008:659–666.
74. Kanazawa K, Matsumoto R, Imamura H, et al. Intracranially-recorded ictal direct current shifts may precede high frequency oscillations in human epilepsy. *Clin Neurophysiol* 2015;126:47–59.
75. Usui N, Terada K, Baba K, et al. Significance of very-high-frequency oscillations (over 1,000 Hz) in epilepsy. *Ann Neurol* 2015;78:295–302.
76. Ibrahim GM, Wong SM, Anderson RA, et al. Dynamic modulation of epileptic high frequency oscillations by the phase of slower cortical rhythms. *Exp Neurol* 2014;251:30–38.
77. Nonoda Y, Miyakoshi M, Ojeda A, et al. Interictal high-frequency oscillations generated by seizure onset and eloquent areas may be differentially coupled with different slow waves. *Clin Neurophysiol* 2016;127:2489–2499.
78. Guirgis M, Chinvarun Y, Del Campo M, et al. Defining regions of interest using cross-frequency coupling in extratemporal lobe epilepsy patients. *J Neural Eng* 2015;12:026011.