Original article

*Epileptic Disord 2015; 17 (4): 413-24*

# Interictal high-frequency oscillations (HFOs) as predictors of high frequency and conventional seizure onset zones

Pradeep Modur, Svjetlana Miocinovic

University of Texas Southwestern Medical Center, Dallas, TX, USA

Received January 17, 2015; Accepted June 27, 2015

**ABSTRACT** – We investigated the relationship between the interictal high-frequency oscillations (HFOs) and the seizure onset zones (SOZs) defined by the ictal HFOs or conventional frequency activity (CFA), and evaluated the usefulness of the interictal HFOs as spatial markers of the SOZs. We analysed seizures showing discrete HFOs at onset on intracranial EEGs acquired at ≥1000-Hz sampling rate in a training cohort of 10 patients with temporal and extratemporal epilepsy. We classified each ictal channel as: HFO+ (HFOs at onset with subsequent evolution), HFO- (HFOs at onset without evolution), CFA (1.6-70-Hz activity at onset with evolution), or non-ictal. We defined the SOZs as: hSOZ (HFO+ channels only), hfo+&-SOZ (HFO+ and HFO- channels), and cSOZ (CFA channels). Using automated methods, we detected the interictal HFOs and extracted five features: density, connectivity, peak frequency, log power, and amplitude. We created logistic regression models using these features, and tested their performance in a separate replication cohort of three patients. The models containing the five interictal HFO features reliably differentiated the channels located inside the SOZ from those outside in the training cohort ($p<0.001$), reaching the highest accuracy for the classification of hSOZ. Log power and connectivity had the highest odds ratios, both being higher for the channels inside the SOZ compared with those outside the SOZ. In the replication cohort of novel patients, the same models differentiated the HFO+ from HFO- channels, and predicted the extents of the hSOZ and hfo+&-SOZ (F1 measure >0.5) but not the cSOZ. Our study shows that the interictal HFOs are useful in defining the spatial extent of the SOZ, and predicting whether or not a given channel in a novel patient would be involved in the seizure. The findings support the existence of an abnormal network of tightly-linked ictal and interictal HFOs in patients with intractable epilepsy.

**Key words:** high-frequency oscillations, epilepsy, intracranial EEG, seizure localization, seizure prediction

**Correspondence:**
Pradeep Modur
Department of Neurology & Neurotherapeutics,
5323 Harry Hines Blvd,
Dallas, TX 75390, USA
<pmodur@gmail.com>

doi:10.1684/epd.2015.0774

For presurgical localization in epilepsy, the seizure onset zone (SOZ) is commonly used as an indirect measure of the theoretical epileptogenic zone (EZ), although the correlation between the two may not always be accurate (Rosenow and Lüders, 2001). Although evidence suggests that the SOZ defined by the ictal high-frequency oscillations (HFOs) or infraslow activity may be superior to that defined by the conventional frequency activity (CFA) (Modur *et al*., 2012), it does not obviate the need for obtaining ictal recordings. While the concept of being able to determine the EZ based on the interictal epileptiform discharges is attractive, its practical utility has not materialized. However, recent studies have shown that the pathological HFOs are a promising alternative in defining the EZ. The interictal HFOs (*i.e*. ripples: 80-200 Hz; and fast ripples: 250-600 Hz) have been shown to localize the seizure focus, correlate with the surgical outcome, and serve as markers for epileptogenicity and impending seizures (Jirsch *et al*., 2006; Jacobs *et al*., 2009; Zijlmans *et al*., 2009; Wu *et al*., 2010; Akiyama *et al*., 2011; Zijlmans *et al*., 2011; Pearce *et al*., 2013; Wang *et al*., 2013). Similarly, the ictal HFOs have been shown to localize the SOZ, participate in seizure genesis and propagation, and correlate with the surgical outcome (Allen *et al*., 1992; Fisher *et al*., 1992; Alarcon *et al*., 1995; Worrell *et al*., 2004; Akiyama *et al*., 2005; Ochi *et al*., 2007; Modur and Scherg, 2009; Modur *et al*., 2011; Fujiwara *et al*., 2012; Modur *et al*., 2012; Park *et al*., 2012). These studies have led to the HFOs being now proposed as a new electrophysiological biomarker for epilepsy (Engel and da Silva, 2012; Zijlmans *et al*., 2012).

Although the research related to interictal HFOs is encouraging, the published literature is limited to the investigation of the variations in the firing rate of ripple and fast ripple HFOs with respect to the SOZ defined by the CFA (1-70 Hz). Based on this, it was reported that good seizure outcome was associated with the resection of tissue corresponding to the interictal HFOs rather than the extent of the resection itself or the resection of the tissue corresponding to the CFA-defined SOZ or the conventional interictal spikes (Jacobs *et al*., 2010; Akiyama *et al*., 2011; Haegelen *et al*., 2013). The clinical applicability of these studies is rather limited because:

– the threshold HFO "firing rate" at which the tissue is considered epileptogenic is not easily quantifiable (Jacobs *et al*., 2010);

– the "interictal HFO zone" can be widespread, extending for several centimetres, particularly in neocortical epilepsy (Crépon *et al*., 2010).

Thus, it would be impractical to plan resection based on the interictal HFOs alone without considering the extent of the SOZ. Furthermore, the rationale for relying on the interictal HFOs rather than the SOZ to define the surgical boundary is even more debatable in the context of our recent study showing that a prospectively-determined, ictal HFO-defined SOZ can be much smaller than the CFA-defined SOZ, and that its resection can also lead to a favourable seizure outcome (Modur *et al*., 2011). These results prompted us to hypothesize the existence of an HFO-based epileptic network in which the interictal HFOs are more closely linked spatially to the HFO-defined SOZ rather than the CFA-defined SOZ.

In the current study, we aimed:

– to test the above hypothesis by investigating the relationship between the interictal HFOs and the two SOZs;

– to examine the usefulness of the interictal HFO as spatial markers of the SOZ.

Using automated HFO detection and feature extraction methods, we developed the logistic regression models in a training cohort of patients, and tested the performance of the models in predicting the extent of the SOZ in a separate replication cohort of novel test patients. Our study draws attention to the finer differentiation between the high frequency and conventional SOZs with potentially important clinical implications for surgical epilepsy therapies.

## Materials and methods

### Patient population

Two cohorts of patients were recruited for the study: a training cohort for developing the logistic regression models and a replication cohort for testing the models. All patients were evaluated according to a standard presurgical protocol to determine the need, type of electrodes, and extent of coverage for intracranial monitoring. The inclusion criteria were:

– intracranial recordings acquired with a 1000-Hz sampling rate or higher;

– well-defined electrical seizure onset preceding the clinical seizure onset;

– at least one seizure showing discrete HFOs at onset (>70-Hz activity, ≥400-ms duration).

We excluded patients for whom adequate interictal data were not archived. Informed consent was obtained from all patients.

### Intracranial recordings

The EEGs for the training and replication cohorts were acquired on the 128-channel Nihon-Kohden (Nihon-Kohden, Foothill Ranch, CA) and Stellate (Natus Inc., San Carlos, CA) systems, respectively. The sampling rate of 1000 Hz allowed reliable visualization of activity up to 333 Hz. The implanted electrodes

consisted of subdural grids and strips (contact diameter: 2.3 mm; intercontact distance: 10 mm), intracerebral depth electrodes (4-8 contacts per electrode; contact diameter: 1.1 mm; intercontact distance: 10 mm), or both, obtained from the same manufacturer (Ad-Tech Medical, Racine, WI).

### Definition of SOZs

The method of determining the SOZ is described in detail elsewhere (Modur *et al*., 2011). Briefly, the clinical seizure onset was first marked by the earliest observable behavioural change on the video. Second, the occurrence of clear rhythmic activity in the CFA range was marked using bipolar montages, 1.6-70-Hz bandpass with 60-Hz notch filtre, and 10-s/page window; conventional electrographic onset was then defined by tracing backwards to identify the timing of the earliest occurrence of discrete ≤70-Hz rhythmic activity. Third, the ictal HFO-based SOZ was defined by identifying all the channels that showed discrete HFOs within a 2-s window around the time of conventional seizure onset using bipolar montages, 53-300-Hz bandpass with 60-Hz notch filtre, and 2-s/page window (*figure 1*). The HFO channels were then classified as HFO+ (channels that showed subsequent evolution of activity) or HFO- (channels that did not show evolution). In other words, a channel was classified as HFO+ when clear-cut HFO activity (>70 Hz) was seen at seizure onset, followed by continuation of the HFO activity or its evolution into the CFA (≤70 Hz) in the same channel until the appearance of the first behavioural change (*figure 2*). Multiple seizures were analysed for each patient, and if a given channel was classified as HFO+ for one seizure but HFO- for another seizure, its final designation was HFO+. HFO+ channels from all the seizures were combined to determine the definitive SOZ. Thus, by visual inspection, we classified each channel during the seizure as: HFO+, HFO-, CFA, or non-ictal. The ictal *vs*. non-ictal and HFO+ *vs*. HFO- classifications were mutually exclusive, however, an HFO+ or

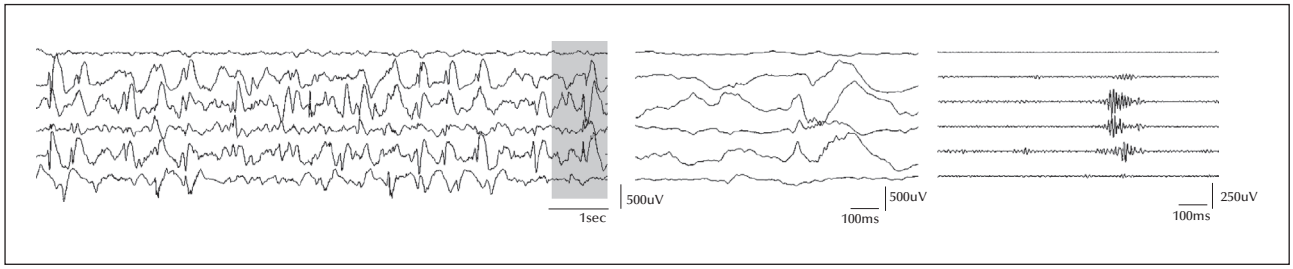


**Figure 1.** Visual identification of the interictal high-frequency oscillations (HFOs). Left panel: at the conventional setting of 1.6-70 Hz and 10 seconds per page, the highlighted segment shows a clear spike. Middle panel: changing the time base to 2 seconds per page with the same filtre settings shows rhythmic oscillations suggestive of HFOs. Right panel: at the high frequency setting of 50-300 Hz and 2 seconds per page window, the presence of discrete HFOs is confirmed. Note the entire highlighted segment in the left panel is expanded in the middle and right panels.

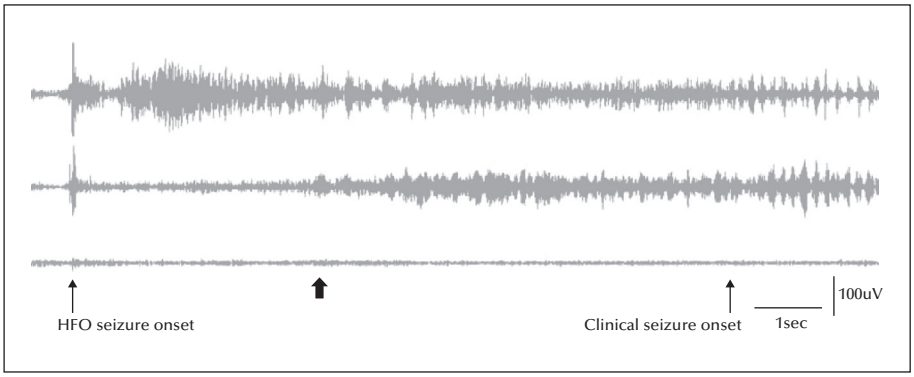


**Figure 2.** Determination of the seizure onset zone (SOZ) based on the ictal high-frequency oscillations (HFOs). At the high frequency setting of 50-300 Hz, rhythmic HFOs are seen at seizure onset in the top and middle channels (ictal HFO channels) but not in the bottom channel ("HFO seizure onset" arrow). Initially, the ictal HFOs evolve in the top channel (*i.e*. HFO+) but not in the middle channel (*i.e*. HFO-) which becomes involved a few seconds later (short arrow). At the time of appearance of clinical symptoms ("clinical seizure onset" arrow), the top and middle channels remain involved in the seizure, with evolution of ictal HFOs into the slower, conventional frequency activity (CFA). In this example, the top channel would be classified as HFO+ and CFA, the middle channel as HFO- and CFA, and the bottom channel as non-ictal.

HFO- channel could also be classified as CFA (*figure 2*). Based on the channel classification, we defined the SOZs as follows: hSOZ (comprised of HFO+ channels only), hfo+&-SOZ (comprised of both HFO+ and HFO- channels), and cSOZ (comprised of CFA channels).

**Automated detection of interictal HFOs**

Raw unfiltered EEG recordings were uploaded into MATLAB 2011b (MathWorks, Natick, MA) after converting the native vendor file formats to the EDF+ format. After visual inspection using the bipolar montages, those channels that were heavily contaminated by artefact were excluded from further analysis. The EEG was bandpass-filtered at 50-333 Hz using the Butterworth 6-order zero-phase digital filtre. Notch filtre was applied to remove the 60-Hz line noise and its harmonics. The Hilbert transform was then calculated for each channel (*supplementary figure 1*). A threshold function, 2 standard deviations above the mean Hilbert signal, was applied to identify the segments that would qualify as HFOs. Only the segments that were ≥9-ms long and contained at least six peaks (two of which being ≥4 standard deviations above the mean rectified EEG signal) were considered as candidate HFOs, and retained for further analysis. Segments that were <14-ms apart were combined into a single detection. A candidate HFO segment was classified as a definite HFO if its mean frequency was >70 Hz or its total power in the 70-333-Hz frequency range was greater than the total power in the <70-Hz frequency range (calculated by the fast Fourier transform [FFT]). For segments >512-ms long, the FFT was performed on partial segments of data using 512-ms width and 50% window overlap. If any sub-segment satisfied the criteria for an HFO, then the entire segment was classified as an HFO. All segments detected as definite HFOs along with those that were considered as HFOs but ultimately rejected were separately identified and displayed (*supplementary figure 2*). We tried several different combinations of energy functions (Hilbert transform, root mean square, and line length) and threshold parameters (standard deviation and Tukey inner and outer fence) as reported by others (Staba *et al*., 2002; Gardner *et al*., 2007; Akiyama *et al*., 2011) but the most consistent and reliable HFO detections were obtained using the Hilbert transform with the settings described above. We recognized the possibility of detecting the so-called "false HFOs" (*supplementary figure 3*) caused by the filtering of sharp artefacts (Bénar *et al*., 2010); we minimized such detections by visually removing the sections of data with signal discontinuities and persistent sharp artefacts, and by mandating that the detected HFOs contain at least six rectified peaks above the mean signal threshold.

**Validation of the detection algorithm**

Two experienced epileptologists validated the performance of the algorithm. Rater 1 participated in the algorithm development whereas rater 2 did not. Each rater evaluated a one-minute data segment in two randomly-selected, high-firing and low-firing channels, in five patients. A custom graphical user interface allowed the raters to scroll through the data, one channel at a time, in one-second epochs. The raters were asked to indicate if the computer-marked segments represented the HFOs (including both the definitive and putative HFOs), and were also given the opportunity to freely designate any additional segments that should have been designated as HFOs that the computer missed. Using this method, the algorithm had 77-91% sensitivity, 96-98% specificity, and 93-97% accuracy between the two raters. The concordance between the computer and the raters was "substantial" (kappa: 0.68; rater 2) or "perfect" (kappa: 0.84; rater 1).

**Selection of HFO features**

We empirically identified five features to characterize the interictal HFOs: density, connectivity, peak frequency, log power, and amplitude. Density (ms/min) is a measure of the amount of HFOs present in a channel given by the sum of all HFO durations/number of minutes of data analysed. Because the HFO rate can be influenced by variations in the algorithms in the handling of the HFOs of different duration (Staba *et al*., 2002; Pearce *et al*., 2013), we chose the density which combines both the rate and duration. Connectivity (number of connections per minute) is a measure of the network synchrony for a given channel, defined as the sum of HFO events occurring simultaneously between that channel and other channels divided by the number of channels sharing the simultaneous HFO events, normalized by the amount of data analysed. This is a simpler version of the connectivity measures described by other investigators (van Diessen *et al*., 2013), and is given by:

$$\frac{1}{N} \cdot \frac{1}{t} \sum_{i,j=1, j \neq i}^{N-1} HFO_{ij}$$

where $HFO_{ij}$ is an HFO event occurring simultaneously between channels $i$ and $j$, $N$ is the total number of channels, and $t$ is the total time analysed in minutes. The HFOs in two channels were considered simultaneous if they started or ended within 10 ms of each other. Peak frequency (Hz) is the mean of peak frequencies of all the HFOs in a given channel. Rather than analysing the frequency in different bands (Staba *et al*., 2002; Blanco *et al*., 2010), we relied on the peak frequency of each HFO because higher peak frequency

was found to be a differentiating factor for the HFO types (Modur *et al*., 2011). Log power ($\mu V^2$) is the mean of the logarithm of the average power of the HFOs in the 70-333-Hz band. We selected this because higher peak power was a characteristic of HFO+ in our prior study (Modur *et al*., 2011). Amplitude ($\mu V$) is the mean of the average amplitude of the HFOs, and represents another feature similar to the power.

### Extraction of HFO features

We extracted the HFO features from the archived data from a training cohort of 10 patients with temporal and extratemporal epilepsy. For each patient, we analysed a 20-minute interictal file consisting of discontinuous data segments of variable length (each 30-60 seconds), sampled intermittently (every 15-30 minutes) for several hours over multiple days. The number and dose of the antiepileptic drugs varied when the interictal recordings were obtained. The archived data did not allow for the awake and sleep state distinction. Using the automated method, we detected the HFOs in each channel and extracted the features. We compared each feature inside *vs*. outside the various SOZs (*i.e*. hSOZ, hfo+&-SOZ, and cSOZ) and between the HFO types (HFO+ *vs*. HFO-) using the Mann-Whitney U-test in GraphPad Prism (GraphPad Software, La Jolla, CA). We considered $p$ values $<0.05$ as significant.

### Logistic regression models for spatial and temporal classification

We created the binomial logistic regression (BLR) models based on the HFO features derived from the training cohort in SPSS (IBM Corporation, Armonk, NY). For the spatial classification of channels, we entered all the five features into the model with SOZ as the dependent variable, and performed the following comparisons: hSOZ *vs*. non-hSOZ channels; hfo+&-SOZ *vs*. non-hfo+&-SOZ channels; cSOZ *vs*. non-cSOZ channels; and HFO+ *vs*. HFO- channels. To measure the strength of association between the predicted probabilities and the classification, we plotted the receiver operating characteristic (ROC) curves for each feature and for all the features considered together. We constrained the classification of the SOZ channels to have high specificity by selecting the cut-off point on the ROC curve that corresponded to the sensitivity at the 0.8 specificity on the x-axis.

### Evaluation of prediction performance

We tested the prediction performance of the BLR models in a separate replication cohort of three patients. For reproducibility, we collected two interictal files for each patient from different days, each file 20 minutes long, consisting of a single continuous data segment (unlike the training interictal file which was discontinuous). To test the model performance in the replication cohort, we used the regression coefficients derived from the training cohort to obtain the probabilities of prediction. From a practical standpoint, our goal was to test the applicability of the group-level models to predict in a novel test patient whether or not a given channel will be classified as a SOZ channel *before* the first seizure occurred. Once the first seizure occurs in the test patient, the SOZ channels will be known, limiting the further utility of the group-level models unless a second, independent SOZ exists.

We assessed the prediction performance using the F1 measure, which is the harmonic mean of recall (*i.e*. sensitivity) and precision (*i.e*. positive predictive value), given by:

$$F1 = \frac{2 \cdot R \cdot P}{R + P}$$

where $R$ (recall) is given by $\frac{TP}{TP+FN}$ and $P$ (precision) is given by $\frac{TP}{TP+FP}$; $TP$: true positives, $FN$: false negatives, and $FP$: false positives. The value of the F1 measure ranges from 0 to 1, with 1 suggestive of best performance.

## Results

Among 24 consecutive patients who underwent intracranial monitoring, 10 patients met the inclusion criteria for the training cohort. Fourteen patients were excluded because of: inadequate data sampling rate ($n$=5), lack of seizures during monitoring ($n$=2), or insufficient archived interictal data ($n$=7). For the replication cohort, we identified three recent consecutive patients (*table 1*). Patients 1-5 were included in our previous study (Modur *et al*., 2011). The median number of channels analysed was 83 (range: 59-100) in the training cohort and 76 (range: 55-94) in the replication cohort. Among 822 available channels in the training cohort, 15 were excluded due to persistent artefacts, leaving 807 channels for analysis: 211 HFO+, 159 HFO-, 307 CFA, and 130 non-ictal channels. Among 228 available channels in the replication cohort, three were excluded due to persistent artefacts, leaving 225 channels for analysis: 49 HFO+, 34 HFO-, 72 CFA, and 70 non-ictal channels.

### Characteristics of interictal HFOs

The interictal HFO features inside the SOZ differed significantly from those outside the SOZ (*figure 3, supplementary table 1*). The values of density, connectivity, amplitude, and log power were *higher* inside the SOZs (hSOZ, hfo+&-SOZ, and cSOZ) than

**Table 1.** Characteristics of patients in the training and replication cohorts.

| Pt | Age / Sex | Epilepsy duration (years) | MRI | Engel outcome | Electrodes implanted (n) | Channels analysed, (n) | Intracranial recording sites | Intracranial seizure onset zone | Channel classification | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | HFO+ (n) | HFO- (n) | CFA (n) |
| 1 | 32/M | 30 | Normal | III | 88 | 66¶ | Left: F-P convexity, PF, MF, LT Right*: MF, LF | Left frontal | 29 | 24 | 40 |
| 2 | 26/F | 22 | Normal | II | 128 | 84 | Right: F-P convexity, OF, MF, LT, MT, ST, insula Left*: MF, LF | Right inferior and lateral temporal | 4 | 27 | 11 |
| 3 | 19/F | 6 | Right parietal FCD and nodular heterotopia | I | 96 | 82 | Right: P-O convexity, LT, ST, MO | Right parieto-occipital | 27 | 34 | 54 |
| 4 | 20/M | 17 | Normal | II | 124 | 99 | Left: F-P convexity, PF, MF, LT Right*: MF, LF | Left frontal | 35 | 30 | 52 |
| 5 | 19/M | 12 | Normal | I | 128 | 87¶ | Right: F-P convexity, LT, ST, LP, LO, MO | Right posterior temporal-occipital | 46 | 6 | 51 |
| 6 | 38/M | 36 | Right MTS; subtle left MTS | I | 96 | 77 | Right: F-P convexity, LT, MT, ST Left*: MT | Right inferomesial and lateral temporal | 12 | 4 | 9 |
| 7§ | 19/F | 17 | Left MTS | I | 84 | 73 | Left: F-T convexity, LT, MT, ST | Left inferomesial temporal | 12 | 11 | 18 |
| 8 | 51/M | 8 | Normal | I | 116 | 95 | Left: F-P convexity, PF, LT, MT Right*: MT | Left mesial and lateral temporal | 6 | 13 | 15 |
| 9 | 18/M | 15 | Bilateral hippocampal signal hyperintensity | I | 72 | 59 | Right: LF, LT, MT Left*: LT, MT | Right mesial and lateral temporal | 11 | 10 | 23 |
| 10 | 41/M | 40 | Right P-O EM; right MTS; | I | 124 | 100 | Right: F-P convexity, LO, LT, MT Left*: MT | Right posterior temporal-parietal; independent right anterolateral temporal | 29 | 0 | 34 |

**Table 1.** (*Continued*)

| Pt | Age / Sex | Epilepsy duration (years) | MRI | Engel outcome | Electrodes implanted (n) | Channels analysed, (n) | Intracranial recording sites | Intracranial seizure onset zone | Channel classification | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | HFO+ (n) | HFO- (n) | CFA (n) |
| 1R | 23/M | 19 | Subtle left MTS | III | 108 | 94 | Left: F-P convexity, OF, LT, MT, ST Right*: LT | Left lateral temporal | 8 | 2 | 23 |
| 2R | 37/M | 22 | Left perisylvian heterotopia | I | 88 | 76¶ | Left: F-P convexity, PF, LT, ST, intralesional | Left parietal | 31 | 25 | 38 |
| 3R | 54/F | 37 | Normal | I | 68 | 55¶ | Right: LT, ST, LO, LP | Right lateral temporal | 10 | 7 | 11 |

Training cohort included Patients 1-10. Replication cohort included Patients 1R-3R.
§Reoperation after failed prior left selective amygdalohippocampectomy. *Limited coverage. ¶Implantation included only subdural electrodes (note that all other patients had both subdural and intracerebral depth electrodes).
FCD: focal cortical dysplasia; EM: encephalomalacia; MTS: mesial temporal sclerosis; HFO: high-frequency oscillation; CFA: conventional frequency activity; F-P: fronto-parietal; P-O: parieto-occipital; PF: prefrontal; MF: medial frontal; LF: lateral frontal; OF: orbitofrontal; LP: lateral parietal; LT: lateral temporal; MT: mesial temporal; ST: subtemporal; LO: lateral occipital; MO: medial occipital; LP: lateral parietal.

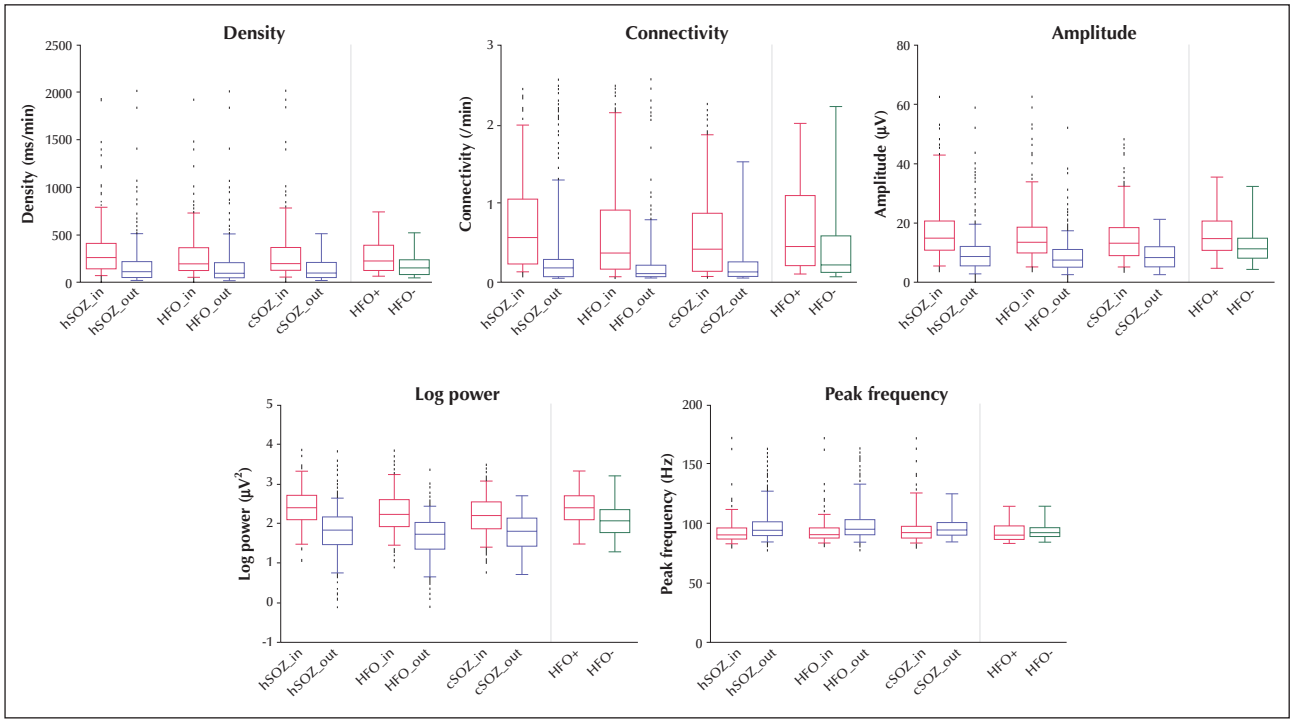


**Figure 3.** Characteristics of the interictal high frequency oscillations (HFOs). Results of Mann-Whitney U test are shown for the five features (density, connectivity, peak frequency, log power, and amplitude) for the spatial classification of channels as inside *vs*. outside the various seizure onset zones (SOZs). The bottom and top edges of each box correspond to the 25$^{th}$ and 75$^{th}$ percentile values respectively; the bottom and top whiskers correspond to the 5$^{th}$ and 95$^{th}$ percentile values respectively; the horizontal line within the box represents the median; dots on either side of the whiskers represent the outliers. All the differences were significant ($p<0.05$). hSOZ_in: inside hSOZ; hSOZ_out: outside hSOZ; HFO_in: inside hfo+&-SOZ; HFO_out: outside hfo+&-SOZ; cSOZ_in: inside cSOZ; cSOZ_out: outside cSOZ; HFO+: ictal HFOs with evolution; HFO-: ictal HFOs without evolution. See text for details.

outside, whereas the peak frequency was *lower* inside the SOZs than outside ($p<0.0001$). Density and connectivity showed 2-4-fold differences in the absolute values, being higher inside than outside the SOZs. The HFOs in the HFO+ channels showed *higher* density, connectivity, amplitude, and log power ($p<0.0001$) but *lower* peak frequency ($p<0.05$) than those in the HFO- channels.

### Spatial classification of the SOZs

In the training cohort, the model containing all the five interictal HFO features reliably differentiated the channels located inside the SOZ from those outside the SOZ ($p<0.001$). The respective accuracy and ROC area under curve (AUC) of the group models were: 81% and 0.81 for the classification of hSOZ; 76% and 0.82 for the classification of hfo+&-SOZ; 70% and 0.75 for the classification of cSOZ; and 62% and 0.69 for the classification of HFO+ *vs.* HFO- channels (*figure 4*). Individually, the log power and connectivity were the most useful differentiating features. Log power had the odds ratio (OR) of 20.5, 14.5, and 11.8 for the classification of hSOZ, hfo+&-SOZ, and cSOZ, respectively. Connectivity had the odds ratio (OR) of 1.7, 5.9, and 1.6 for the classification of hSOZ, hfo+&-SOZ, and cSOZ, respectively.

### Prediction of the SOZs

We tested the performance of the classification models of the training cohort by applying them to the replication cohort consisting of novel patients. The F1 measures showed some variability across patients but were largely similar in each patient within a given category (*figure 5, supplementary table 2*). Analysis of the two datasets for each patient showed that the classifier was able to differentiate the HFO+ channels from the HFO- channels in all three patients (F1 0.57-0.86), predict the hfo+&-SOZ channels in three patients (F1 0.50-0.86), and predict the hSOZ channels in two patients (F1 0.57-0.69). However, the classifier was not reliable in predicting the cSOZ channels (F1 0.27-0.53).

## Discussion

In this study, we investigated the relationship between the interictal HFOs and the two SOZs defined by the ictal HFOs or conventional frequencies, and evaluated the usefulness of the interictal HFOs as spatial markers of the SOZs. Using automated HFO detection and feature extraction methods, we developed the logistic regression models in a training cohort of patients, and tested their prediction performance in a separate replication cohort of novel patients. The main findings were:

– the interictal HFOs inside the SOZ differed significantly from those outside the SOZ;

– the interictal HFO features derived from the training cohort predicted with reasonable certainty whether or not a given channel in a novel test patient would be involved in a seizure with ictal HFOs before the occurrence of the first seizure.

The implications of these findings are discussed below.

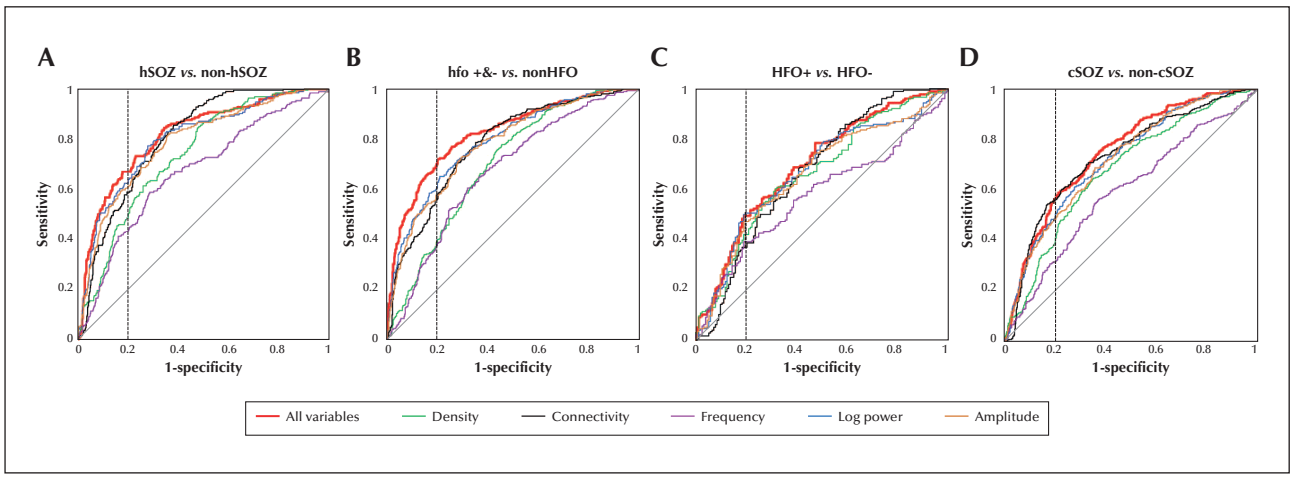


**Figure 4.** Receiver operating characteristic (ROC) curves for the spatial classification of channels. The ROC curves correspond to the group-level logistic regression models in the training cohort. The curves are obtained from the interictal high-frequency oscillation (HFO) features individually and together for the spatial classification of channels localized to inside *vs.* outside the different seizure onset zones (hSOZ, hfo+&-SOZ, and cSOZ), and for the differentiation of HFO+ *vs.* HFO- channels. The dashed, vertical line corresponds to the 0.8 specificity cutoff.

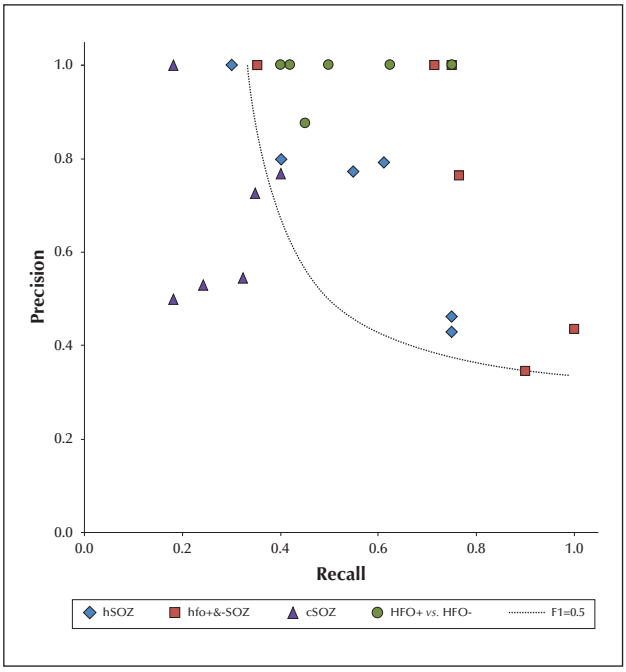


**Figure 5.** F1 measures for prediction of the seizure onset zone. The datasets from the three patients in the replication cohort are shown. The datasets indicated by the plotted values above and to the right of the dotted line, which corresponds to F1=0.5, were considered to have been classified reliably. See text for details.

### Interictal HFOs as spatial predictors of the SOZ

Our study shows that multiple HFO features can differentiate the SOZ channels from the non-SOZ channels, with higher classification accuracy for the HFO-defined SOZs than the cSOZ. The HFO density in a given channel was several folds higher inside the SOZ but was not a discriminating feature in the logistic regression analysis. This finding differs from other studies that showed that the HFO firing rate (equivalent to the density) was a discriminating feature (Bragin *et al*., 2002; Staba *et al*., 2002; Jacobs *et al*., 2008, 2009; Andrade-Valença *et al*., 2012). This discrepancy could be related to the methodological differences in estimating the two closely related features. We found the HFO connectivity among the channels to be a differentiating feature between the SOZ and non-SOZ channels. This has not been reported before, and it points to the presence of a tightly linked HFO network inside the SOZ, likely reflecting underlying epileptogenicity. The log power was a discriminatory feature for the SOZ channels but the amplitude (similar to the power) was not. This is consistent with other studies showing higher spectral power of the HFOs inside the SOZ (Matsumoto *et al*., 2013; Pearce *et al*., 2013), and suggests that the HFO morphology is useful for localizing the SOZ. At a sampling rate of 1000 Hz, the median frequency range of the HFOs in our study was 90-95 Hz, which is similar to the ripple frequency range reported by others (Cho *et al*., 2014). Although some investigators have evaluated HFO frequencies up to 600 Hz and shown that the fast ripples are tightly linked to the SOZ (Bragin *et al*., 2002, Staba *et al*., 2002, Cho *et al*., 2014), others were unable to record fast ripples (Crépon *et al*., 2010). Interestingly, the peak HFO frequency was lower inside the SOZ in our study. This is not surprising as the pathological HFOs, which are likely to be prevalent inside the SOZ, have been shown to have lower mean frequencies than the physiological HFOs (Matsumoto *et al*., 2013). Our findings, in conjunction with others, support the notion that the interictal HFO rate, morphology, and network properties are useful spatial markers of the SOZ, particularly the HFO-defined SOZ.

Application of the group models from the training cohort to the replication cohort yielded reliable prediction of the spatial extent of hfo+&-SOZ in all three patients, however, the model predicted the extent of hSOZ in two patients, and was largely unsuccessful in predicting the extent of cSOZ. The poor performance of our models in predicting the extent of cSOZ is not surprising because the CFA-defined SOZ is felt to be spatially different from the ictal HFO-defined SOZ (Modur *et al*., 2011). To our knowledge, this is the first report demonstrating a tight link between the interictal HFOs and the ictal HFO-defined SOZ rather than the CFA-defined SOZ. Our prior work showed that:

– the ictal HFOs can be widespread at seizure onset but evolve subsequently with different characteristics, *i.e*. HFOs+ are higher in peak frequency and peak power, and spatially restricted in contrast to the HFO-;

– the seizure onset defined by the HFO+ occurs earlier, and in a significantly different and smaller distribution than the seizure onset defined conventionally;

– the resection of the HFO+ channels based on a prospectively-defined protocol can result in favourable outcome (Modur *et al*., 2011).

The current study, in conjunction with our prior study, points to the existence of an "epileptic HFO network" which seems to be expressed not only ictally but also interictally. The neurophysiological difference between the HFO-defined SOZ and the CFA-defined SOZ remains unclear but it is possible that they represent activity from different generators (Jiruska *et al*., 2010).

### Interictal HFOs as predictors of the ictal HFO type

We found that the interictal HFO features were able to differentiate the HFO+ channels from the HFO- channels with a moderate degree of accuracy. Application of the group models from the training cohort to the replication cohort also yielded reliable

differentiation of the HFO+ and HFO- channels in all three patients. This is particularly relevant in the context of our recent report that the resection of HFO+ channels can be associated with favourable surgical outcome (Modur *et al*., 2011). It also strengthens the notion of an "epileptic HFO network" mentioned above, and provides a valuable method for localizing the more clinically meaningful type of the ictal HFO-defined SOZ.

Our study has a few limitations. We were limited by the discontinuous nature of the interictal data in the training set. Continuous data segments would have been desirable to construct more robust models. Because of the preset sampling rate in our data set, we were unable to evaluate the interictal HFOs >333 Hz (*i.e*. fast ripples) which have been found to be useful by other investigators. The lower sampling rate could also have resulted in lower peak frequencies of the HFOs observed in this study. It is still unclear how to differentiate the physiological HFOs (*i.e*. associated with cognitive and motor tasks) from the pathological HFOs (*i.e*. epileptiform HFOs described in this study) (Matsumoto *et al*., 2013). It is possible that some physiological HFOs may have been detected by our algorithm. Because the interictal data were obtained in the same manner in all patients and in all states, it is unlikely that such detections would have seriously impacted the results. Furthermore, the finding of HFOs with higher log power and lower peak frequency inside the SOZ in our study provides reassurance that the pathological HFOs were more likely to have been detected than the physiological HFOs (Matsumoto *et al*., 2013). Finally, the small sample size of our study suggests the need for further validation in a larger cohort.

In summary, our study highlights the ability of the interictal HFOs to define the spatial extent of the SOZ, and to predict with reasonable certainty whether or not a given channel in a novel patient would be involved in the seizure (particularly with the HFO+ type activity) before the first seizure occurs. This holds promise for patients who fail to have seizures despite chronic invasive monitoring or those who cannot tolerate it for longer periods. Our findings also suggest a tight linkage between the interictal HFOs and the HFO-defined SOZ. As the pathological HFOs are shown to be specific to epilepsy (Modur, 2014), and felt to be generated by mechanisms that are distinct from those of the conventional EEG activity (Jiruska *et al*., 2010), an understanding of the epileptic HFO network should enhance our knowledge of epileptogenesis and ictogenesis. Presence of such a network would argue for placing the implantable devices for epilepsy treatment (*i.e*. closed-loop responsive neurostimulation device [Morrell, 2011] and seizure prediction system [Cook *et al*., 2013]) in close proximity to the HFO-defined SOZ. Because the interictal HFOs are more tightly linked to the HFO-defined SOZ as shown in this study, knowledge of the spatial extent of the interictal HFO network may prove to be beneficial in tailoring the location of these devices. □

**Supplementary data.**

Summary didactic slides and supplementary figures and tables are available on the www.epilepticdisorders.com website.

**Acknowledgements and disclosures.**

We thank Dr. Song Zhang, PhD, Department of Clinical Sciences (Biostatistics), UT Southwestern Medical Center, for his assistance in statistical analysis. We thank Dr. Ryan Hays, MD, Department of Neurology, UT Southwestern Medical Center, for his assistance in the validation of the software for automated detection of HFOs.

Research reported in this study was supported in part by the National Center for Advancing Translational Sciences of the National Institutes of Health under award number UL1TR001105. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

None of the authors have any conflict of interest to disclose.

## References

Akiyama T, Otsubo H, Ochi A, *et al*. Focal cortical high-frequency oscillations trigger epileptic spasms: confirmation by digital video subdural EEG. *Clin Neurophysiol* 2005; 116: 2819-25.

Akiyama T, McCoy B, Go CY, *et al*. Focal resection of fast ripples on extraoperative intracranial EEG improves seizure outcome in pediatric epilepsy. *Epilepsia* 2011; 52: 1802-11.

Alarcon G, Binnie CD, Elwes RD, *et al*. Power spectrum and intracranial EEG patterns at seizure onset in partial epilepsy. *Electroencephalogr Clin Neurophysiol* 1995; 94: 326-37.

Allen PJ, Fish DR, Smith SJ. Very high-frequency rhythmic activity during SEEG suppression in frontal lobe epilepsy. *Electroencephalogr Clin Neurophysiol* 1992; 82: 155-9.

Andrade-Valença L, Mari F, Jacobs J, *et al*. Interictal high frequency oscillations (HFOs) in patients with focal epilepsy and normal MRI. *Clin Neurophysiol* 2012; 123: 100-5.

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: 301-10.

Blanco JA, Stead M, Krieger A, *et al*. Unsupervised classification of high-frequency oscillations in human neocortical epilepsy and control patients. *J Neurophysiol* 2010; 104: 2900-12.

Bragin A, Wilson CL, Staba RJ, *et al*. Interictal high-frequency oscillations (80-500 Hz) in the human epileptic brain: entorhinal cortex. *Annal Neurol* 2002; 52: 407-15.

Cho JR, Koo DL, Joo EY, *et al*. Resection of individually identified high-rate high-frequency oscillations region is associated with favorable outcome in neocortical epilepsy. *Epilepsia* 2014; 55: 1872-83.

Cook MJ, O'Brien TJ, Berkovic SF, *et al*. Prediction of seizure likelihood with a long-term, implanted seizure advisory system in patients with drug-resistant epilepsy: a first-in-man study. *Lancet Neurol* 2013; 12: 563-71.

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.

Engel Jr. J, da Silva FL. High-frequency oscillations – where we are and where we need to go. *Prog Neurobiol* 2012; 98: 316-8.

Fisher RS, Webber WR, Lesser RP, *et al*. High-frequency EEG activity at the start of seizures. *J Clin Neurophysiol* 1992; 9: 441-8.

Fujiwara H, Greiner HM, Lee KH, *et al*. Resection of ictal high-frequency oscillations leads to favorable surgical outcome in pediatric epilepsy. *Epilepsia* 2012; 53: 1607-17.

Gardner AB, Worrell GA, Marsh E, *et al*. Human and automated detection of high-frequency oscillations in clinical intracranial EEG recordings. *Clin Neurophysiol* 2007; 118: 1134-43.

Haegelen C, Perucca P, Châtillon CE, *et al*. High-frequency oscillations, extent of surgical resection, and surgical outcome in drug-resistant focal epilepsy. *Epilepsia* 2013; 54: 848-57.

Jacobs J, LeVan P, Chander R, *et al*. Interictal high-frequency oscillations (80-500 Hz) are an indicator of seizure onset areas independent of spikes in the human epileptic brain. *Epilepsia* 2008; 49: 1893-907.

Jacobs J, Levan P, Chatillon CE, *et al*. High frequency oscillations in intracranial EEGs mark epileptogenicity rather than lesion type. *Brain* 2009; 132: 1022-37.

Jacobs J, Zijlmans M, Zelmann R, *et al*. High-frequency electroencephalographic oscillations correlate with outcome of epilepsy surgery. *Annal Neurol* 2010; 67: 209-20.

Jirsch JD, Urrestarazu E, LeVan P, *et al*. High-frequency oscillations during human focal seizures. *Brain* 2006; 129: 1593-608.

Jiruska P, Powell AD, Chang WC, *et al*. Electrographic high-frequency activity and epilepsy. *Epilepsy Res* 2010; 89: 60-5.

Matsumoto A, Brinkmann BH, Matthew Stead S, *et al*. Pathological and physiological high-frequency oscillations in focal human epilepsy. *J Neurophysiol* 2013; 110: 1958-64.

Modur P. High frequency oscillations and infraslow activity in epilepsy. *Ann Indian Acad Neurol* 2014; 17: 99-106.

Modur PN, Scherg M. Intracranial broadband EEG analysis and surgical outcome: case report. *Clin Neurophysiol* 2009; 120: 1220-4.

Modur PN, Zhang S, Vitaz TW. Ictal high-frequency oscillations in neocortical epilepsy: implications for seizure localization and surgical resection. *Epilepsia* 2011; 52: 1792-801.

Modur PN, Vitaz TW, Zhang S. Seizure localization using broadband EEG: comparison of conventional frequency activity, high-frequency oscillations, and infraslow activity. *J Clin Neurophysiol* 2012; 29: 309-19.

Morrell MJ. Responsive cortical stimulation for the treatment of medically intractable partial epilepsy. *Neurology* 2011; 77: 1295-304.

Ochi A, Otsubo H, Donner EJ, *et al*. Dynamic changes of ictal high-frequency oscillations in neocortical epilepsy: using multiple band frequency analysis. *Epilepsia* 2007; 48: 286-96.

Park S-C, Lee SK, Che H, *et al*. Ictal high-gamma oscillation (60-99 Hz) in intracranial electroencephalography and postoperative seizure outcome in neocortical epilepsy. *Clin Neurophysiol* 2012; 123: 1100-10.

Pearce A, Wulsin D, Blanco JA, *et al*. Temporal changes of neocortical high frequency oscillations in epilepsy. *J Neurophysiol* 2013; 110(5): 1167-79.

Rosenow F, Lüders H. Presurgical evaluation of epilepsy. *Brain* 2001; 124: 1683-700.

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: 1743-52.

van Diessen E, Otte WM, Braun KPJ, *et al*. Improved diagnosis in children with partial epilepsy using a multivariable prediction model based on EEG network characteristics. *PLoS ONE* 2013; 8: e59764.

Wang S, Wang IZ, Bulacio JC, *et al*. Ripple classification helps to localize the seizure-onset zone in neocortical epilepsy. *Epilepsia* 2013; 54: 370-6.

Worrell GA, Parish L, Cranstoun SD, *et al*. High-frequency oscillations and seizure generation in neocortical epilepsy. *Brain* 2004; 127: 1496-506.

Wu JY, Sankar R, Lerner JT, *et al*. Removing interictal fast ripples on electrocorticography linked with seizure freedom in children. *Neurology* 2010; 75: 1686-94.

Zijlmans M, Jacobs J, Zelmann R, *et al*. High-frequency oscillations mirror disease activity in patients with epilepsy. *Neurology* 2009; 72: 979-86.

Zijlmans M, Jacobs J, Kahn YU, *et al*. Ictal and interictal high frequency oscillations in patients with focal epilepsy. *Clin Neurophysiol* 2011; 122: 664-71.

Zijlmans M, Jiruska P, Zelmann R, *et al*. High-frequency oscillations as a new biomarker in epilepsy. *Annal Neurol* 2012; 71: 169-78.

## TEST YOURSELF

(1) What types of EEG activity can be seen in intracranial recordings obtained at a sampling rate of 1000 Hz?

(2) Which of the following features of the interictal high-frequency oscillations (HFOs) were most useful in differentiating the channels located inside the seizure onset zone (SOZ) from those outside the SOZ in this study?

(3) The interictal HFOs most reliably predicted the spatial extent of which of the following seizure onset zones in this study?

*Note: Reading the manuscript provides an answer to all questions. Correct answers may be accessed on the website, www.epilepticdisorders.com, under the section "The EpiCentre".*