■ Original article

Epileptic Disord 2021; 23 (2): 299-312

Epileptic Disorders

Effects of a stable concentration of propofol on interictal high-frequency oscillations in drug-resistant epilepsy

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Received March 12, 2020;
Accepted November 08, 2020

* Portions of this work were presented as a pilot study in the Proceedings at the 51st Annual Congress of the Japanese Epilepsy Society held in Kyoto, Japan, on November 4 2017 and at the 71st American Epilepsy Society Annual Meeting held in Washington DC, United States of America, on December 2, 2017.

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doi:10.1684/epd.2021.1264

ABSTRACT

Objective. The aim of this study was to clarify the effect of a stable concentration of propofol on interictal high-frequency oscillations (HFOs), which may contribute to identifying the epileptogenic zone intraoperatively for resection surgery.

Methods. Nine patients with drug-resistant focal epilepsy who underwent invasive pre-surgical evaluation with chronic subdural electrodes were recruited. Five-minute electrocorticograms during wakefulness, slow-wave sleep, and under a stable brain concentration of propofol were recorded with the same electrodes. In each patient, 1-10 pairs of electrodes were selected for both electrodes with EEG changes within 5 seconds from the ictal onset (ictal pattern for 5 seconds [IP5]) and those outside the area of IP5 with no interictal epileptiform discharges (non-epileptiform [nEPI]). The numbers of ripples (80-250 Hz) and fast ripples (>250 Hz) were measured semi-automatically using an established algorithm. Statistical testing was performed with a mixed effect model.

Results. Thirty-seven pairs of electrodes from nine patients were analysed for IP5 and 29 pairs from seven patients were analysed for nEPI. The numbers of HFOs differed between the areas (IP5 and nEPI) and among the conditions (wakefulness, slow-wave sleep, propofol anaesthesia) (all $p < 0.01$). The HFO occurrence rates were significantly higher for IP5 than those for nEPI in all conditions (for both ripples and fast ripples in all conditions; $p < 0.01$).

Significance. The occurrence rates of HFOs for IP5 were significantly higher than those for nEPI under propofol anaesthesia. These are fundamental findings for intraoperative HFO analysis, however, the following limitations should be considered: physiological HFOs could not be completely differentiated from pathological HFOs; in order to apply an HFO detector, an appropriate cut-off threshold is needed; an artefact of the impulse response filter appears as an HFO; and the series was comprised of a small number of heterogeneous patients.

Key words: high-frequency oscillations, propofol anaesthesia, electrocorticogram, intraoperative recording, epileptogenic zone

In recent years, the presence of interictal high-frequency oscillations (HFOs) has been recognized as one of the possible biomarkers of epileptogenicity [1, 2]. HFOs were first recorded using microelectrodes to study neuronal mechanisms underlying epileptogenic properties of human mesial temporal lobe (MTL) structures [3, 4]. Soon afterwards, it became apparent that ictal and interictal HFOs could be recorded even with clinical macroelectrodes [5-8], and that HFOs recorded with microelectrodes and those recorded with macroelectrodes differ in terms of their distribution and their frequencies, though both are associated with epileptic activity [8, 9]. By using intracranial macroelectrodes, the relationship between the seizure onset zone (SOZ) and HFOs has been investigated [1, 10]. It has been reported that the surgical removal of HFO-generating tissues leads to a good postoperative outcome [2, 11, 12], and that withdrawal of anti-epileptic drugs (AEDs) increased both epileptic seizures and the occurrence of HFOs, implying a tight link between epileptic seizures and HFOs [13]. Although HFOs have been considered clinically useful for detecting the SOZ, detecting HFOs is sometimes difficult in real-world clinical practice. Visual HFO detection requires training and is very time-consuming. To solve these issues, some groups developed automatic HFO detectors [10, 14-16]. The occurrence rate of HFOs also depends heavily on the patient's vigilance. Using intracranial macroelectrodes, Bagshaw *et al*. reported that the rate of HFOs was highest in non-rapid eye movement (nREM) sleep and lower in wakefulness and rapid eye movement (REM) sleep [17]. Regarding the effects of sedative drugs on HFOs, some previous reports noted that the occurrence rate of HFOs increased after propofol cessation, probably reflecting its anticonvulsant effect [18]. Propofol is a short-acting intravenous agent used for induction and maintenance of general anaesthesia, and it has two opposite properties, anti-convulsive and pro-convulsive [19]. How interictal HFOs behave under a stable blood concentration of propofol has not been elucidated. In addition, whether the HFOs during propofol anaesthesia (induced sleep state) behave like those during natural sleep (physiological sleep state) has not been investigated.

The aim of the present study was to clarify the effect of a stable concentration of propofol on interictal HFOs. Based on a hypothesis that the occurrence rate of HFOs under a stable concentration of propofol differs from that during wakefulness and natural sleep state, a semi-automated algorithm was applied to the electrocorticogram (ECoG) data acquired from electrodes associated with epileptogenicity and those unrelated to epileptogenicity in patients with drug-resistant focal epilepsy. Validating the hypothesis may contribute to the development of a less invasive method for the identification of the epileptogenic zone intraoperatively.

Methods

Patients

A total of 13 patients who underwent chronic subdural electrode implantation for pre-surgical evaluation at the Kyoto University Hospital between January 2014 and December 2017 were initially recruited. Of these, four patients whose intraoperative ECoGs were not available due to poor recording conditions were excluded. Therefore, nine patients (six males and three females; mean age: 34.4 years; age range: 16 to 61 years) were finally enrolled. Three patients had mesial temporal lobe epilepsy (MTLE), and the other six had neocortical epilepsy; two with frontal lobe epilepsy, two with parietal lobe epilepsy, one with temporal lobe epilepsy, and one with fronto-temporo-parietal lobe epilepsy. We refer to the electrodes showing the earliest ECoG changes (within five seconds from ictal onset of ECoG) in habitual seizures as "ictal pattern for 5 seconds (IP5)" based on 14 days of ECoG recording, except for Patient 4. Since Patient 4 did not have a spontaneous epileptic seizure even on withdrawal of AEDs and one stimulus-induced seizure was recorded during the study of single-pulse electrical stimulation at 1 Hz, the stimulated electrodes and the electrodes showing the earliest ECoG changes (within five seconds) in the stimulus-induced seizure were exceptionally defined as IP5 in this patient. On the contrary, the electrodes outside the area of IP5 and those with no interictal epileptiform discharges were referred to as "non-epileptiform (nEPI)". In four patients, electrodes were placed on the occipital lobe for the ECoG recording during chronic implantation, although these electrodes were not analysed because the occipital lobe generates frequent physiological HFOs [20]. All electrodes that met the above criteria of IP5 and nEPI were analysed. While the ECoGs for IP5 were recorded in all nine patients, those for nEPI were recorded only in seven patients due to the immediate and wide spread of the ictal discharge (Patient 6 had fronto-temporo-parietal epilepsy with epileptic spasms) and the limitation of intraoperative recording channels (Patient 5 had MTLE). There were no electrodes that met the criteria for nEPI in these two patients. The clinical details of the patients and the number of electrode pairs and their implantation sites are shown in *table 1*. The patient with poor clinical outcome (Patient 6) with epileptic spasms showed immediate spread of the ictal discharge, which implied insufficient coverage of the SOZ. His epileptogenic zone could be a deeply seated lesion and thus his first ictal activities were seen on the frontal cortex and immediately, after about 300 milliseconds, propagating to the temporal and the parietal cortices. However, we assumed that the electrodes considered for IP5 might

▼ **Table 1.** Patient profile.

Pt No.	Age /Gender	Epilepsy /Pathology	Area of IP5 (no. of electrodes)	No. of resected electrodes for IP5	Area of nEPI (no. of electrodes)	TCI (µg/ml)/ETCO2 (mmHg)/ BIS/dose of remifentanil (µg/kg/min)	Engel classification	Follow-up period (months)	Sleep staging by R&K
1	61/ M	Lt. PLE/ Oligoastrocytoma	Lt. PoCG, IPL (4)	4	Lt. IPL (2)	2.4/ 44/ 53/ 0.1	1	72	-
2	39/ M	Rt. MTLE /HS + FCD 1A	Rt. PHG (1)	1	Rt. PHG, FG, ITG (4)	3.0/ 38 /38/ 0.1	1	65	+
3	29/ M	Rt. FLE /FCD 1A	Rt. MFG, IFG (3)	3	Rt. MFG, SFG (2)	3.0/ 41/ 48/ 0.1	2	15	+
4	16/ F	Lt. FLE /DNET	Lt. PreCG (2)	2	Lt. SFG (4)	3.0/ 30/ 40/ 0.1	1	58	+
5	41/ M	Lt. MTLE /HS + FCD 1A	Lt. PHG, FG, STG, ITG, IPL (7)	5	na	2.8/ 33/ 45/ 0.3	1	48	-
6	17/ M	Rt. F-T-PLE /FCD 1A	Rt. MFG, IFG, STG IPL /(10)	10	na	3.0/ 39/ 60/ 0.3	3	41	-
7	23/ F	Lt. PLE /FCD 2B	Lt. PoCG (3)	3	Lt. STG, MTG, SFG, MFG, IPL (6)	3.0/ 36/ 58/ 0.1	1	34	+
8	34/ M	Lt. MTLE /HS + FCD 1A	Lt. PHG, FG (1)	1	Lt. SFG, MFG, IFG (6)	2.0/ 42/ 45/ 0.05	1	24	+
9	50/ F	Lt. TLE /dual pathology	Lt. STG, MTG, ITG (6)	4	Lt. MFG, IFG, SPL (5)	2.4/ 38/ 63/ 0.1	1	20	-

BIS: Bispectral Index; DNET: dysembryoplastic neuroepithelial tumour; ETCO2: end-tidal carbon dioxide; FCD: focal cortical dysplasia; FG: fusiform gyrus; FLE: frontal lobe epilepsy; IFG: inferior frontal gyrus; IPL: inferior parietal lobule; IP5: ictal pattern for 5 seconds; ITG: inferior temporal gyrus; MFG: middle frontal gyrus; MTG: middle temporal gyrus; MTLE: mesial temporal lobe epilepsy; na: not available; nEPI: non-epileptiform; PHG: parahippocampal gyrus; PLE: parietal lobe epilepsy; PoCG: post central gyrus; PreCG: pre central gyrus; SFG: superior frontal gyrus; STG: superior temporal gyrus; TCI: target control infusion; TLE: temporal lobe epilepsy; R&K: Rechtschaffen&Kales criteria.

be relevant to the epileptogenic zone, at least in part, for the following reasons. First, the epileptic discharges were followed by clinical epileptic spasms. Second, the ictal epileptic activity with epileptic spasms is known to show rapid and wide propagation. Written, informed consent was obtained from all patients and their families. This study protocol was approved by the institutional review board of our institute (No. C725).

Data acquisition

In all patients, subdural electrodes were implanted (platinum, inter-electrode distance of 10 mm, recording surface diameter of 2.3 mm, AD-TECH, Racine, WI, USA; or platinum, inter-electrode distance of 5 or 10 mm, recording surface diameter of 1.5 or 3 mm, Unique Medical Co., Ltd., Tokyo, Japan) and the ECoGs were recorded for 14 days, except for one patient (Patient 4) whose recording period was extended to 19 days because she did not have habitual seizures. ECoGs were referenced to a scalp electrode placed on the skin over the mastoid process contralateral to the side of electrode implantation and sampled at 2,000 Hz with an AC-coupled amplifier with a time constant of 10 or two seconds (0.016 or 0.08 Hz) and an anti-aliasing filter up to 600 Hz (EEG-1100/1200, Nihon Kohden, Tokyo, Japan) for the ECoG recordings during the chronic subdural grid (SDG) implantation. Furthermore, a subgaleal strip electrode facing the galeal layer near the skin incision was implanted as an alternate reference in case the electrodes on the mastoid, used as a reference, contained artefacts in all patients. This was not located close to the suspected SOZ or primary sensorimotor area. Intraoperatively, ECoGs for eight patients were recorded with a 32-channel EEG system (Neuromaster MEE-1232, Nihon Kohden) that consisted of an AC-coupled amplifier with a time constant of two seconds (0.08 Hz), and the sampling rate was set at 2,000 Hz with an anti-aliasing filter up to 600 Hz or at 1,000 Hz with an anti-aliasing filter up to 300 Hz (only in Patient 2).

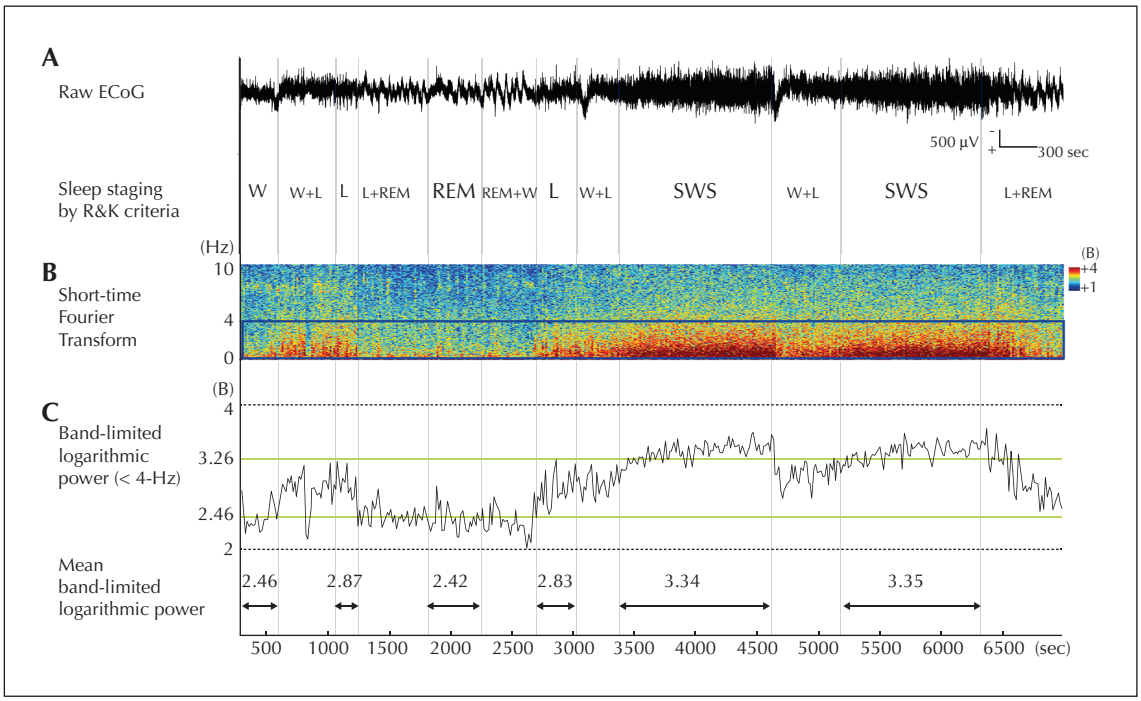
To compare the nature of HFOs between the natural states and the anaesthetic condition, ECoGs were recorded using the same electrodes in the following three conditions: during wakefulness (W), during slow-wave sleep (SWS), and under propofol anaesthesia (Prop). The details of sleep staging are explained in the following subsection. During the recording of ECoGs under propofol, the estimated brain concentration was maintained between 2.0 and 3.0 μg/ml by a target-controlled infusion (TCI). The subdural electrodes were delineated with reference to evoked/related potentials and were co-registered to three-dimensional volume-rendered MRIs, which were reconstructed from MPRAGE (magnetization prepared rapid acquisition with gradient echo) and acquired after electrode implantation. Using the 2D-MRIs, the electrode locations were identified with reference to the major sulci using their signal voids, which were due to the properties of the platinum alloy. These methodological details have been previously described [21].

Sleep staging and epoch selection

The ECoGs for the W and SWS stages were recorded in the latter half of the second week when the dosages of AEDs were returned to preoperative levels. The sleep staging was performed based on the standard Rechtschaffen and Kales (R&K) criteria for five patients. During the ECoG recordings for the sleep staging, scalp EEGs of the central area (C3, C4, Cz, C1, or C2, depending on the location of the surgical scar), including A1 or A2 (ipsilateral to the side of electrode implantation), O1 and O2, two electro-oculograms (EOGs) to monitor eye movements, and two electromyograms on the chin to monitor muscle contraction were simultaneously recorded in these five patients. The sleep stages of these recordings were subsequently judged according to the R&K criteria to further analyse the ECoGs during the W and SWS stages. These methodological details have been reported previously [22].

To perform the sleep staging for the remaining four patients in whom it was not possible to attach the scalp EEG, EOG, and chin electrodes for the sleep staging, the delta band power was analysed. In these four patients, the W stage was determined based on the ECoG and video recordings. For the SWS stage, the power of ECoG was below 4 Hz, which usually increases the most during the SWS stage, was compared between stages. A modified method of He *et al*. was finally applied to the four patients [23]. First, one intracranial electrode located in the frontocentral area that was not affected by epileptiform discharges was selected (*figure 1A*). Then, a time-frequency analysis that ranged below 10-Hz was performed using short-time Fourier Transform (STFT) with a Fourier Time window of 30 seconds and a calculating power spectrum bin of 15 seconds each (half overlapping) (*figure 1B*). The mean <4-Hz band-limited logarithmic power (base 10) of the W and SWS stages was then calculated based on the data of the five patients in whom sleep staging was performed based on the R&K criteria (*figure 1C*). Since the differences in band-limited logarithmic power between the W and SWS stages were 0.8 or more in these five patients, the differences were applied to the sleep staging for the remaining four patients. For example, when the band-limited logarithmic power of the W stage was 1.5, the SWS stage was set in the periods when the band-limited logarithmic power was 2.3 or more.

All interictal ECoG segments used in the HFO analysis were longer than two hours, either before or after any clinical or subclinical seizures. Analyses were



■ **Figure 1.** Methods of sleep staging using band-limited logarithmic power. In this representative patient (Patient 2), sleep stages were defined by the standard Rechtschaffen and Kales (R&K) criteria. (A) A raw electrocorticogram over two hours using a frontocentral electrode did not show any epileptiform discharges. All the sleep stages are presented in this figure. (B) Time-frequency analysis, that ranged below 10-Hz, was performed using Short-Time Fourier Transform (STFT) with a Fourier Time window of 30 seconds and a power spectrum of 15 seconds for each (half overlapping). The definite power values are shown in the logarithmic scale (colour band: 1-4 Bell). A light blue square represents the activities below 4-Hz (<4-Hz) that were subsequently analysed. (C) A time series of a <4-Hz waveform is shown. The vertical axis indicates the logarithmic power (base 10) of the <4-Hz band. The line below in light green indicates the mean band-limited logarithmic power of the W stage, and the line above in light green shows the value of the lower line plus 0.8. At the bottom, the mean band-limited logarithmic power of each stage is shown. During the W and SWS stages, the mean band-limited logarithmic power is 2.46 and 3.34-3.35, respectively. In this patient, the difference in power between the W and SWS stages is 0.88. Similarly, the difference in power between W and SWS stages is 0.8 or more in the other four patients in whom sleep staging was established using R&K criteria (five patients in total). Thus, the value difference of 0.8 between the W and SWS stages was applied to the remaining four patients in whom sleep staging was not established using R&K criteria. W: wakefulness; L: light sleep; REM: rapid eye movement; SWS: slow-wave sleep.

performed on bipolar montages, in association with the two adjacent electrodes.

Anaesthesia management

Recording was performed before the craniotomy, about one hour after anaesthesia induction to avoid the effect of suppression-burst, which may be seen after an intravenous bolus of propofol [24]. None of the patients showed a suppression-burst pattern under propofol. For ECoG recording, the same electrodes were used as during chronic recordings. All nine patients were induced and maintained with total intravenous anaesthesia. General anaesthesia was induced by a continuous drip infusion of propofol using a TCI pump (3.0–8.0 µg/ml) or its bolus infusion (0.6-2.5 mg/kg) and by a continuous drip infusion of remifentanil (0.1-0.3 µg/kg/min). For the maintenance, a continuous drip infusion of propofol using a TCI pump (2.0–3.0 µg/ml) and a low-dose, stable infusion of remifentanil was administered (0.05-0.3 µg/kg/min). A continuous drip infusion of rocuronium bromide (0.2-1.0 mg/kg) was

also used to keep the patients motionless during ECoG recordings. No other sedatives or analgesics were used one hour prior to ECoG recordings. During the recording of ECoGs, the estimated concentration of propofol was in the range of 2.0-3.0 µg/ml, the change in concentration was less than 0.2 µg/ml/min, and the bispectral index (BIS) was within the range of 50 ± 15, suggesting appropriate anaesthesia. End-tidal carbon dioxide was kept within a normo- or hypocarbic range (*table 1*). No other AEDs, except for those usually taken by patients with regular dose, were administered.

HFO detection

HFOs were detected using the semi-automatic HFO detection algorithm modified from the method of von Ellenrieder *et al*. [15, 25].

First, to detect artefacts due to movements, *etc*., the activities recorded on subgaleal electrodes were analysed based on features associated with broadband power increase, especially in the high-frequency bands in the spectrogram, implying the possibility of motion artefacts. An STFT was applied to the periods with an excessive number of artefacts, and those with the least number artefacts were used to calculate logarithmic power below 200 Hz. This STFT was performed using 200 sampling-point Fourier windows (100 ms), resulting in a frequency resolution of 10 Hz (bands centred at DC, 10 Hz, 20 Hz,... and 200 Hz), and the logarithmic power was calculated for each 10-Hz frequency band. Then, the appropriate thresholds to specifically exclude the epochs containing artefacts were determined. ECoGs with logarithmic power larger than the defined thresholds were excluded from subsequent analyses.

Second, in-house Matlab scripts modified from an automatic HFO detection algorithm [15, 25] were applied to five-minute ECoGs for each state (Matlab version 7.12.0, MathWorks Inc., Natick, MA, USA). In brief, this algorithm was used to detect increments of root mean square (RMS) amplitude of the signal in narrow-frequency bands and compare them to the background activity in a five-second sliding window. For this in-house Matlab script, a wide-band and subsequently eight narrow-bands of Finite Impulse Response (FIR) filters were applied. The ripple bands consisted of five bands (80-100, 100-125, 125-160, 160-200, 200 -250 Hz), and the fast ripple bands consisted of three bands (250-315, 315-400, 400-500 Hz). Oscillatory activities greater than +/- 2.5 SD from the baseline and with four or more cycles of the central frequency band were regarded as HFOs. *Figure 2* shows an example of HFOs detected by means of this semi-automatic HFO detection algorithm. For all patients, after automatic detection of HFOs, one-minute subsets of the epochs during the W and SWS stages and all five-minute subsets under propofol were visually reviewed by a board-certified neurosurgeon and neurologist to exclude clear false-positive detections.

Finally, the occurrence rates (events/5 min/pair of electrodes) of ripples (80-250 Hz), and fast ripples (> 250 Hz) were calculated from all pairs of bipolar electrodes for IP5 and nEPI and for each state (W, SWS, and Prop) for all patients. For the low-sampling rate of Patient 2 under propofol anaesthesia, the rate of fast ripples could not be counted. The mean ripple rate was calculated simply by computing across 37 pairs of electrodes for IP5 and 29 pairs of electrodes for nEPI. Similarly, the mean fast ripple rate was calculated simply by computing across 37 (or 36) pairs of electrodes for IP5 and 29 (or 25) pairs of electrodes for nEPI.

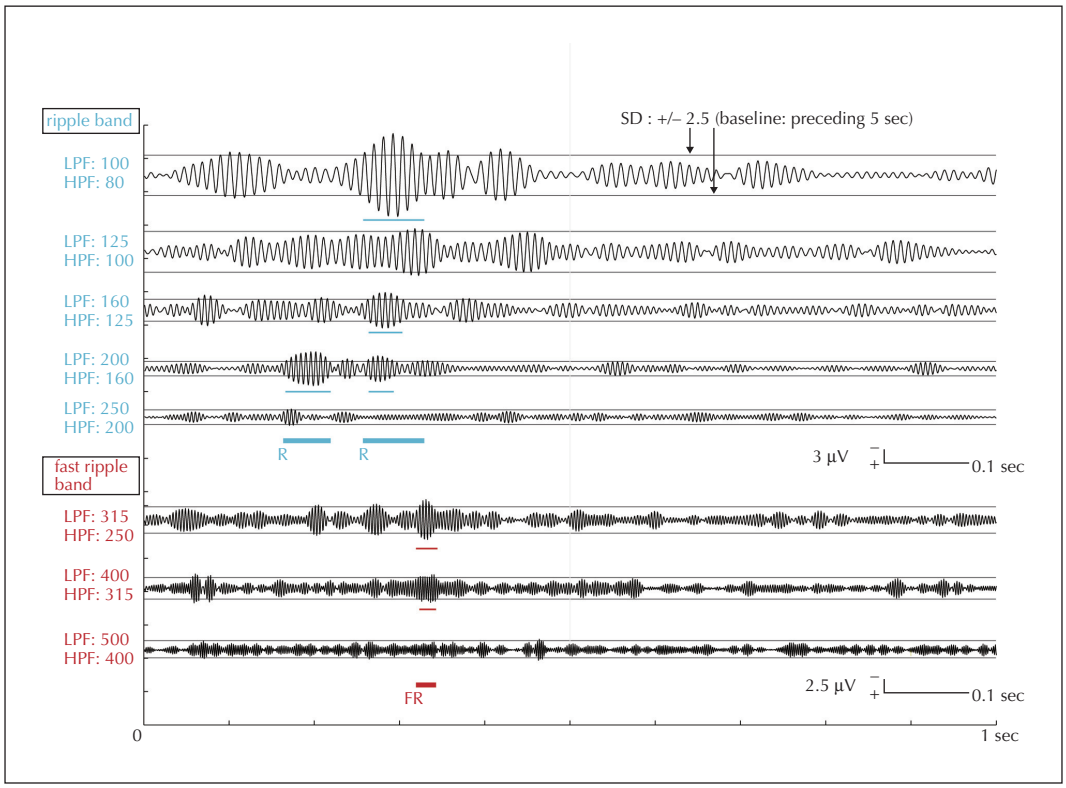
Statistical analysis

Intra- and interindividual variabilities were analysed with linear mixed-models using JMP software (version 12, SAS Institute Inc., Cary, NC, USA). First, to evaluate differences in the occurrence rates of fast ripples and ripples among the three conditions (W, SWS, Prop) for the two areas (IP5 and nEPI), a mixed effect model was applied for fast ripples and ripples separately. Since the areas and conditions had hierarchical relationships in patients, "areas nested within patients" and "conditions nested within patients" were set as fixed effects, and "patients" was set as a random effect. A p-value <0.05 was considered significant. A post-hoc analysis with a mixed effect model was then used to compare between areas (IP5 and nEPI). Because of the heterogeneity of the data, the two patients in whom nEPI electrodes could not be recorded were excluded. "Areas nested within patients" was set as a fixed effect, and "patients" was set as a random effect separately. A p value <0.017 was considered significant for multiple comparisons in the post-hoc analysis. To compare the occurrence rates of HFOs between all pairs of conditions (W vs. SWS, SWS vs. Prop, and W vs. Prop) for IP5 and nEPI, pair-wise comparison was performed using the Tukey-Kramer test.

Results

Differences in the occurrence rate of HFOs

The number of ripples was evaluated from 37 pairs of electrodes in nine patients for IP5 and from 29 pairs of electrodes in seven patients for nEPI (*figure 3A, B*). Except for Patient 2 under propofol anaesthesia, the number of fast ripples was evaluated for the same electrode pairs used for ripples (*figure 4A, B*). The mean HFO rate was determined by simply computing across these pairs of electrodes. For IP5, the occurrence rate of ripples during W (events/5 min/pair of electrodes) was 24.8 ± 5.8 (mean

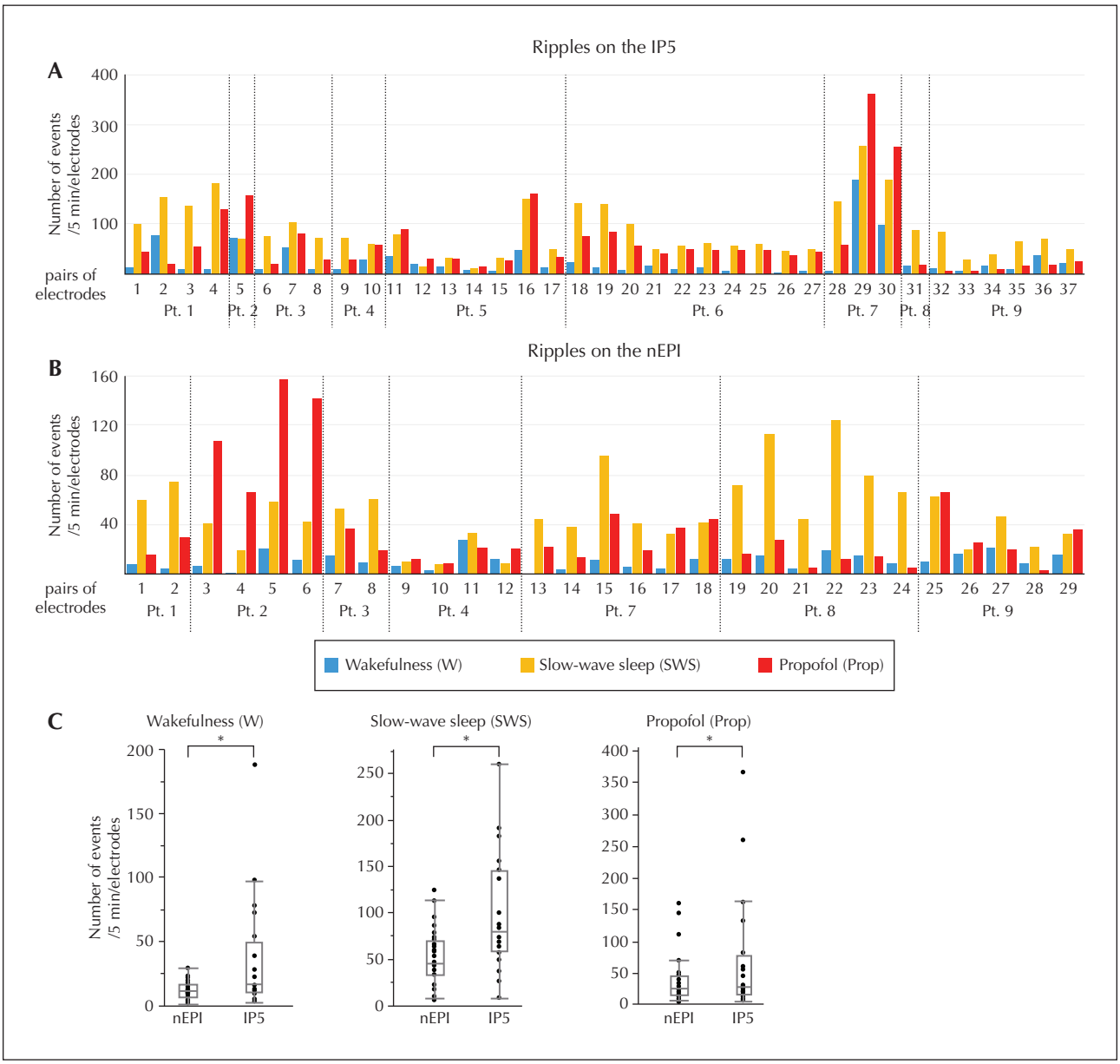


■ **Figure 2.** An example of the use of the automatic HFO detector. A wide band and eight subsequent narrow bands of the Finite Impulse Response (FIR) filter were applied. The ripple band consists of five bands (80-100, 100-125, 125-160, 160-200, 200-250 Hz) and the fast ripple band consists of three bands (250-315, 315-400, 400-500 Hz). Oscillatory activities larger than +/- 2.5 SD of the baseline and with four or more cycles of the central frequency band are detected as HFOs.

± SEM), during SWS was 84.0 ± 9.2, and during Prop was 61.6 ± 11.8. The occurrence rate of fast ripples during W was 7.5 ± 2.4, during SWS was 31.1 ± 5.0, and during Prop was 15.9 ± 5.0. For nEPI, the occurrence rate of ripples during W was 11.1 ± 1.2, during SWS was 50.1 ± 5.3, and during Prop was 36.7 ± 7.1. The occurrence rate of fast ripples during W was 2.8 ± 0.6, during SWS was 7.6 ± 0.9, and during Prop was 4.5 ± 0.8. A mixed effect model yielded significant differences in areas and conditions ($p < 0.01$ for areas and for conditions) with no interaction between areas and conditions, suggesting that both areas and conditions had independent effects on the occurrence rate of HFOs (for both ripples and fast ripples). The Tukey-Kramer test showed that the occurrence rates of ripples and fast ripples were significantly higher in SWS than those in W ($p < 0.017$), whereas there were no differences between SWS and Prop and between Prop and W.

Differences in HFOs between IP5 and nEPI for each condition

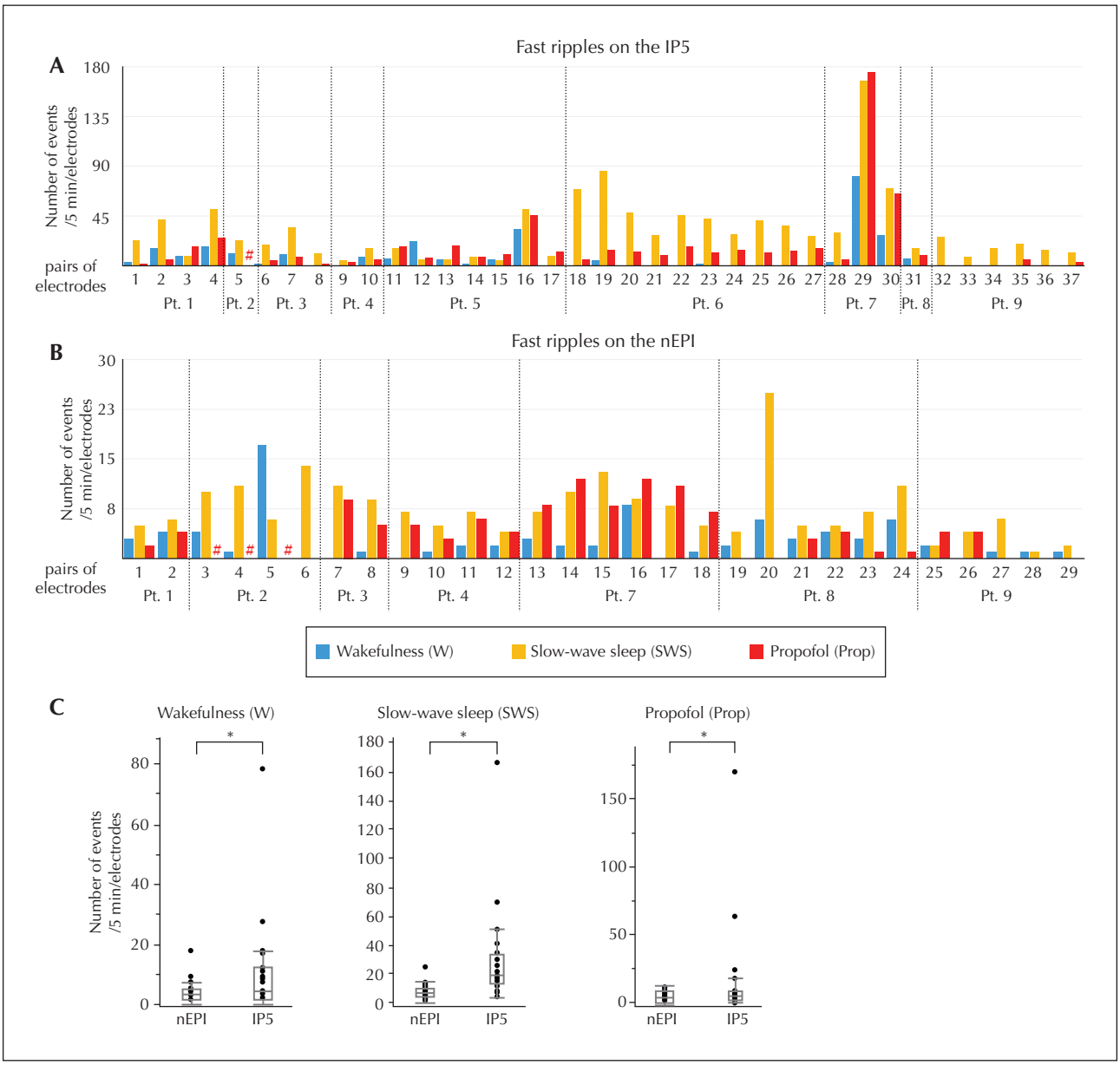
The occurrence rates of the HFOs were compared between IP5 and nEPI for the seven patients based on post hoc analyses performed using a mixed effect model. In all conditions, there were significant differences in ripple occurrence rates ($p = 0.004$ for W and $p < 0.001$ for SWS and Prop) and fast ripple occurrence rates ($p = 0.011$ for W, $p < 0.001$ for SWS, and $p = 0.011$ for Prop) (*figure 3C, 4C*). *Figure 5* shows the receiver operating characteristic (ROC) curves and areas under the curves (AUCs), which demonstrate that fast ripples were associated with a higher AUC value than ripples, implying the slightly higher reliability of fast ripples in detecting IP5 during SWS.



■ **Figure 3.** The occurrence rate of ripples for IP5 and nEPI. The occurrence rate (events per 5 minutes) of ripples from each pair of electrodes among the three conditions (wakefulness [W], slow-wave sleep [SWS], and propofol anaesthesia [Prop]) is shown for IP5 in nine patients (A) and nEPI in seven patients (B). A significant difference in the rate of ripples is shown for areas (IP5/nEPI) and conditions (W/SWS/Prop), independently ($p < 0.001$ for areas and conditions). A mixed effect model was used to compare between IP5 and nEPI for the seven patients, which shows significant differences in all conditions (C *: $p < 0.017$).

Discussion

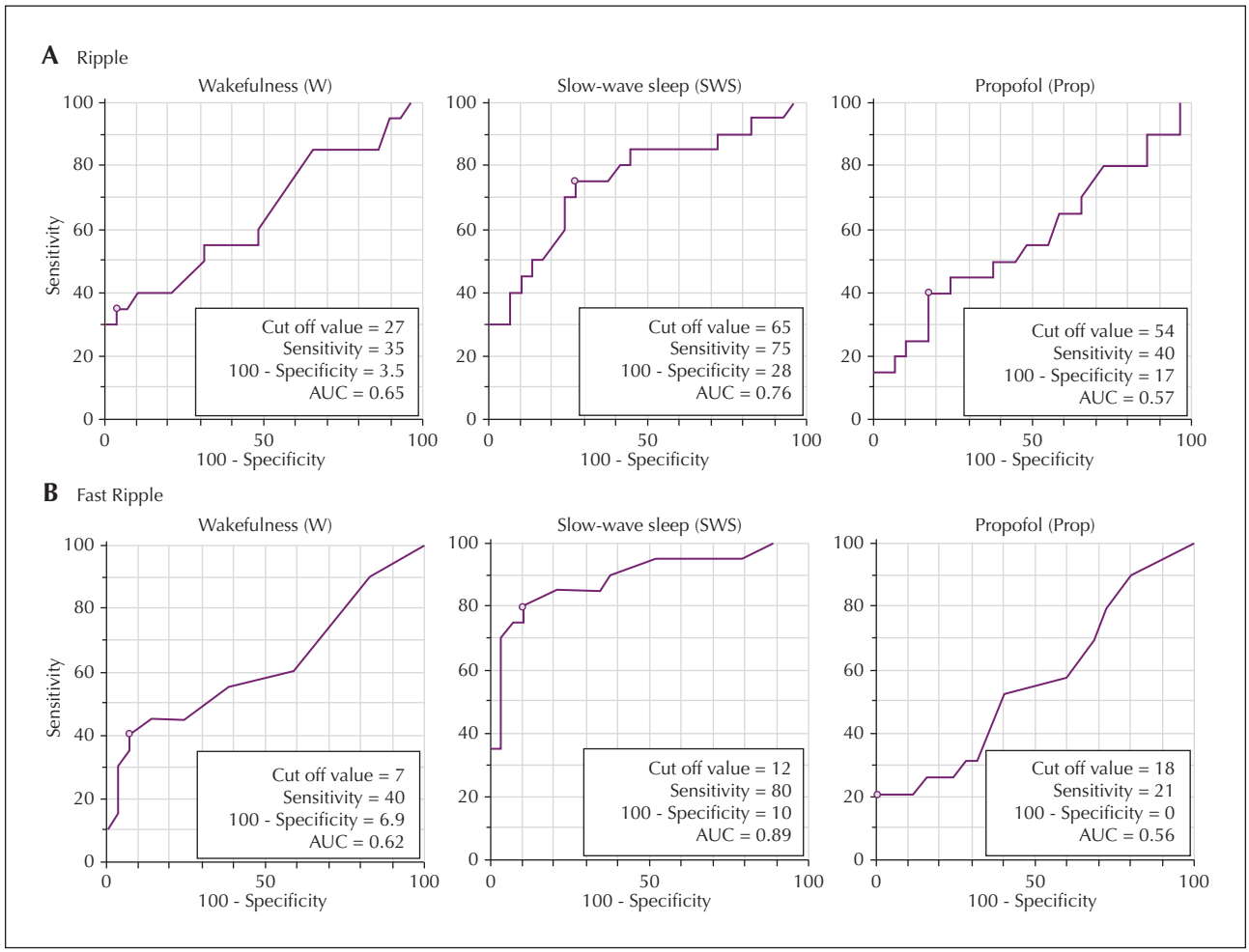
As far as we could ascertain, this is the first study to investigate differences in HFOs under the three different conditions (W, SWS, and Prop) as well as the effect of stable propofol concentration on the occurrence rate of HFOs between areas of epileptogenicity (IP5) and no epileptogenicity (nEPI) in humans using the same electrodes. The present study yielded the following findings: 1) both areas (IP5 and nEPI) and conditions significantly affected the number of HFOs (ripples and fast ripples) independently; and 2) the occurrence rate of HFOs for IP5 was significantly different from that for nEPI under all three conditions.



■ **Figure 4.** The occurrence rate of fast ripples for IP5 and nEPI. The occurrence rate (events per 5 minutes) of fast ripples from each pair of electrodes for the three conditions (wakefulness [W], slow-wave sleep [SWS], and propofol anaesthesia [Prop]) is shown for IP5 in nine patients (A) and nEPI in seven patients (B). A significant difference in the rate of ripples is shown for areas (IP5/nEPI) and conditions (W/SWS/Prop), independently ($p < 0.001$ for areas and $p = 0.007$ for conditions). # data not available. A mixed effect model was used to compare between IP5 and nEPI for the seven patients, which shows significant differences in all conditions (C *: $p < 0.017$).

Occurrence rate of HFOs between conditions and areas

Bagshaw *et al*. reported the spatial relationship between HFOs and SOZ as a function of sleep stages. In their report, spatial specificity was greatest in non-REM stage, and the rate of HFOs was considerably higher in the SOZ than in the non-SOZ [17]. Whereas there were no significant differences between SWS and Prop in the present study, the occurrence rate of HFOs tended to be higher in SWS.



■ **Figure 5.** The receiver operating characteristic (ROC) curves and areas under the curves (AUCs) for ripples and fast ripples in each condition. The AUC during W was 0.65 for ripples and 0.62 for fast ripples, during SWS was 0.76 for ripples and 0.89 for fast ripples, and during Prop was 0.57 for ripples and 0.56 for fast ripples. The optimal cut-off value based on Youden index for ripples and fast ripples was 27 and 7 for W, 65 and 12 for SWS, and 54 and 18 under propofol. Regarding SWS, fast ripples may be more reliable than ripples in detecting IP5 based on the AUCs.

Probable effects of propofol on seizures and EEG

Propofol (2,6-di-isopropylphenol) is a γ-aminobutyric acid (GABA) agonist that activates GABAA receptors directly, inhibits the N-methyl-D-aspartate receptor, and modulates calcium influx through slow calcium ion channels [26]. GABA receptors are macromolecular proteins that form a chloride ion channel complex and contain specific binding sites for GABA and a number of allosteric regulators, including barbiturates, benzodiazepines, and numerous anaesthetic agents [27]. Propofol suppresses seizure activity via GABA-mediated inhibition of neuronal firing by selectively suppressing persistent sodium currents and L-type high-voltage-activated calcium conductance and possibly modulates GABAA receptors at a site different from that targeted by benzodiazepines and barbiturates [28]. Clinically, continuous intravenous infusion of propofol, together with continuous EEG monitoring, is a common mode of treatment for refractory status epilepticus [29, 30].

On the other hand, propofol also has pro-convulsive effects. A potential mechanism for the pro-convulsant properties of propofol may be due to intrinsic subcortical glycine antagonism or GABAergic agonism, as suggested by animal data [31-33]. In non-epileptic

patients, as well as epileptic patients, propofol can induce clinical seizures and seizure-like phenomena [34, 35]. Walder *et al*. reported rates of seizure-like phenomena that occurred during periods of induction (34%), maintenance (3%), emergence (40%), and delay (23%), and assumed that the change in cerebral concentration of propofol was causal [35]. Regarding the EEG, low-dose propofol can induce beta activation or a paradoxical excitation state, whereas high-dose propofol can cause delta and theta power increments [36, 37]. At surgical concentrations under anaesthesia, spindle waves become dominant [38]. This alpha band activity observed during anaesthesia appears to be generated by the same mechanism as that for sleep spindles [39, 40]. Concerning the effects of propofol on HFOs, Zijlmans *et al*. reported that propofol cessation increased the rate of HFOs (both ripples and fast ripples) [18]. They recorded intraoperative ECoGs from TLE patients who did not undergo long-term intracranial recordings with a standardized electrode grid placement. They assumed that the anti-epileptic effects of propofol reduced the number of epileptic HFOs. In the present study, there were no significant differences between SWS and Prop or between Prop and W. Whereas the definitive effects of propofol, *i.e*. pro-convulsive or anti-convulsive, on HFOs cannot be proven in the present study, HFO occurrence rates under a stable concentration of propofol were higher for IP5 than nEPI, with similar effects observed between W and SWS states during chronic electrode implantation.

Bolus infusion of remifentanil can increase the occurrence rates of spikes in a dose-dependent fashion [41]. These pro-convulsive properties of remifentanil might have had some effects on interictal HFOs in the present study, although the dose used in our patients was low (*table 1*).

Clinical implications and limitations

Intraoperative recordings of HFOs can be performed, and are useful for the evaluation of epileptogenicity [42, 43]. In epilepsy surgery, insufficient coverage of chronic subdural electrodes to thoroughly delineate the SOZ can occur mainly due to safety reasons. In contrast, during craniotomy, surgeons can use additional electrodes and evaluate ECoGs over a broader area than during chronic SDG implantation. The present study provides important basic information on the effect of physiological sleep states and anaesthesia on HFOs. We compared the HFOs from electrodes in which ictal activity occurred within five seconds from ictal onset (IP5) and in which no epileptiform discharges were recorded (nEPI). The areas we defined may be larger than those of previous studies, in which the relationship between SOZ and epileptogenicity was investigated, because the five-second interval from the earliest ECoG change was longer in our study than that in the previous studies [7, 8]. Although the epileptogenic zone of Patient 6 with epileptic spasms could reside in deep structures, it was assumed that the analysed electrodes for IP5 might be relevant to the epileptogenic zone, at least in part, as described in the Methods section. Excluding the two patients for whom nEPI could not be recorded, the effect of propofol on the occurrence rate of interictal HFOs was examined by comparing between IP5 and nEPI. The fact that the HFO occurrence rate differed according to the condition of patients provides information about reliability (or unreliability) for each recording situation. In addition, the rapid and semi-automatic HFO detection used in the present study will be helpful intraoperatively, although further development and optimization is required.

There are some limitations to this study. First, whether all HFOs seen for IP5 were purely epileptic was not clear. Previous ECoG studies have provided normative HFO atlases suggesting that, among all non-epileptic sites, the occipital cortex, some temporal regions, or the primary motor and primary somatosensory area generate HFOs [43, 44]. In this study, some analysed electrodes were located on areas known to generate physiological HFOs and high-frequency activity, but electrodes on the occipital lobe, which most frequently generates HFOs, were excluded. It is difficult to completely separate pathological HFOs from physiological HFOs, and some studies have attempted to distinguish between the two by evaluating the coupling of slow waves and HFOs by frequency or phase as one of the characteristics of epileptic HFOs [25, 45]. To focus solely on HFOs, and not the coupling between HFOs and slow activity, as in the present study, the cortices generating physiological HFOs such as the primary motor or sensory cortex should ideally be excluded from the HFO analysis. Because as many electrode pairs as possible were finally adopted, the cortices that may generate physiological HFOs were included in the analyses of both IP5 and nEPI due to the limited number of patients. Further studies of pathological HFOs targeting only the cortices that are unlikely to generate physiological HFOs are expected using larger numbers of patients. Second, the occurrence rate of fast ripples for nEPI in our study was higher than that in other studies in which HFOs were visually identified [7, 8, 17]. The cut-off threshold in the semi-automatic program would be one of the factors that may account for this difference, although the same cut-off threshold was used for all conditions (W/SWS/Prop), areas (IP5/nEPI), and HFOs (ripple/fast ripple). A semi-automatic HFO detection program that was applied in previous studies was used [15, 25].

The HFOs were visually reviewed, as described in the Methods section. Thus, it is unlikely that artefacts were detected as HFOs in the present study. Further studies with an appropriate cut-off threshold are needed from the viewpoint of a trade-off between sensitivity and specificity in applying an HFO automatic detector. Third, when an impulse response filter is applied, the artefacts result in a signal, that appears as an HFO [46]. In the operating room, there are more electrical devices than in the epilepsy monitoring unit, resulting in a high risk of artefact contamination. In the present study, all intraoperative ECoGs were visually confirmed by checking raw waveforms and spectrograms. Although complete automatic detection of HFOs could not be performed, semi-automatic detection, which was considered the best way to exclude the artefacts associated with intraoperative ECoGs, was performed. Finally, the number of patients was small. The high level of heterogeneity of focal refractory epilepsy patients, such as underlying aetiology, localization, or AED usage, is an important factor that may affect the occurrence rate of HFOs. Future larger studies should be performed for comparisons using a homogenous group.

Conclusion

The occurrence rate of HFOs was significantly different between both areas (IP5 and nEPI) and among the different conditions (W, SWS, and Prop). The occurrence rate of HFOs was significantly higher for IP5 than nEPI under a stable concentration of propofol anaesthesia. We believe these findings will support future work in order to establish less invasive epilepsy surgery. ■

Funding.
This work was partly supported by the Japan Ministry of Education, Culture, Sports, Science and Technology (MEXT) KAKENHI Grant Numbers 15H05874, 15K10361, 17K16120, 17K10892, and 19K18424, the Japan Society for the Promotion of Science (JSPS) KAKENHI (grant number 26293209), and research grants from the Japan Epilepsy Foundation and the Japan Brain Foundation.

Acknowledgements and disclosures.
The authors are grateful to Nicolás von Ellenrieder for supporting the automatic HFO detector algorithm.
The authors report no conflicts of interest concerning the materials or methods used in this study or the findings specified in this paper.
The Industry-Academia Collaboration Courses of The Department of Epilepsy, Movement Disorders and Physiology is supported by Eisai Co., Ltd., Nihon Kohden Corporation, Otsuka Pharmaceutical Co., and UCB Japan Co., Ltd. These companies did not play any role in this study, the preparation of the manuscript, or the decision to publish the result.

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TEST YOURSELF

(1) Is there a significant difference in the occurrence rate of HFOs under propofol anaesthesia between electrodes linked to epileptogenicity (ictal pattern for 5 seconds [IP5]) and those that are not (non-epileptiform [nEPI])?

(2) Does a stable concentration of propofol increase the rate of interictal high-frequency oscillations (HFOs)?

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".