Detection of seizure-associated high-frequency oscillations above 500 Hz

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Summary

High-frequency oscillations (HFOs) of up to 500 Hz in EEG are considered to have close relation with ictogenesis. We had the unique opportunity to record a seizure in EEG with intracerebral macroelectrodes and a sampling frequency of 10 kHz. Considering the notion that faster HFOs are likely more ictogenic, we investigated this ictal EEG data to find if even faster HFOs were present.

HFOs were investigated in interictal spikes and seizure activity using time–frequency spectra: *t* values corresponding to frequencies from 100 to 1000 Hz were obtained by comparison to the background and controlled by the false discovery rate (FDR).

The seizure had a right hippocampal onset. HFOs up to 800 Hz as well as HFOs below 500 Hz built up in the hippocampal discharges more at the beginning of the seizure and during the preictal period than in the interictal period. These HFOs were visually confirmed in temporally expanded EEG traces.

We demonstrated for the first time the existence of HFOs above 500 Hz and up to 800 Hz with intracerebral macroelectrodes in an epileptic patient; they occurred primarily in association with the seizure discharge. HFOs above 500 Hz possibly reflect facilitation of ictogenic neuronal hypersynchronization.

Keywords

High-frequency oscillation; Mesial temporal lobe epilepsy; Ictal EEG; Time–frequency analysis; False discovery rate

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Conflict of interest

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

Introduction

Recording of high-frequency brain activity ranging from 100 to 10,000 Hz was pioneered in the era of paper EEG and oscillography using experimental animals (Rodin et al., 1973; Rodin, 2005). Now with digital EEG recording, high-frequency oscillations (HFOs) are attracting attention because of their close relation with epileptogenesis (Allen et al., 1992; Le Van Quyen et al., 2006). HFOs ranging from 80 to 250 Hz (ripples) are recorded from the hippocampus and entorhinal cortex of normal rodents (Buzsáki et al., 1992; Chrobak and Buzsáki, 1996) and also from human hippocampus (Bragin et al., 1999). In kainic acid-treated rats and patients with mesial temporal lobe epilepsy, HFOs of 250–500 Hz (fast ripples) have been detected from regions close to the ictogenic lesion, and fast ripples are thought to indicate pathological hypersynchronous events strongly associated with seizure generation (Bragin et al., 1999, 2002; Staba et al., 2002; Rampp and Stefan, 2006).

Fast ripples were initially detected using microelectrodes or microwires, but Jirsch et al. (2006) showed that HFOs up to 500 Hz can be recorded from human epileptic patients during focal seizures using depth macroelectrodes. Their investigation was limited by their sampling frequency of 2 kHz with a low-pass filter at 500 Hz.

We had a unique opportunity to record a seizure originating from the hippocampus with depth macroelectrodes and a sampling frequency of 10 kHz. Considering the notion that faster HFOs are likely more ictogenic, we investigated this ictal EEG data to find if even faster HFOs were present. Although a single seizure was available for analysis, we expect that the results would prompt advancement of analysis of HFOs in EEG.

Materials and methods

Patient and EEG recording

A 29-year-old man with medically intractable mesial temporal lobe epilepsy was recorded with intracranial electrodes using a Nihon-Kohden (Tokyo, Japan) Neurofax at the Okayama University Hospital. He had bi-temporal depth electrodes and additional sub-dural electrodes manufactured by Unique Medical (Tokyo, Japan). The depth electrodes were stereotactically implanted targeting the amygdalae and hippocampi using Medtronic (Minneapolis, USA) StealthStation® treatment guidance system, and had 6 platinum electrode contacts (numbered 1 through 6 from tip). The surface area of each contact was 3.6 mm², and the inter-contact interval was 5 mm with respect to contacts 1–4, 15 mm between contacts 4 and 5, and 5 mm between contacts 5 and 6. With respect to the subdural electrodes, the surface area of each contact was 7.1 mm² and their inter-contact interval was 10 mm.

The EEG was recorded mostly with a sampling frequency of 1000 Hz to have a sufficient number of seizures captured. During a period between completion of the ordinary monitoring and the scheduled surgery, the EEG was recorded with a sampling frequency of 10 kHz and a band-pass filter ranging from 0.016 Hz to 3 kHz. Because recording with this high sampling frequency is very demanding for the system, the Neurofax allowed usage of only the first 18 electrodes of the system and at most 30 min recording; in this case, the first 18 electrodes included the right depth electrodes and the subdural electrodes placed over the

basal part of the right temporal lobe, and could not be arbitrarily arranged to include electrodes in the contralateral hemisphere. The patient happened to have his habitual complex partial seizure originating from the right hippocampus during this brief recording session (Fig. 1). The patient was free from seizures for the post-surgical follow-up period of 1 year and 6 months after right selective amygdalohippocampectomy.

This study has been approved by the Okayama University Ethics Committee. Informed consent for the study was obtained from the patient.

Spectral analysis

Spectral analysis was performed in the ictal EEG data recorded with a sampling frequency of 10 kHz in the bipolar derivations at the mesial end of each bundle with the greatest seizure involvement (RA1–RA2, RH1–RH2, and RB1–RB2 at the right amygdala, hippocampus and parahippocampal gyrus, respectively) (Fig. 1). We used the bipolar derivations because we expected very local effects; in addition, the electrodes used in the 10 kHz recording did not include an electrode appropriate for a reference. The ictal rhythmic activity was separated into 13 morphologically different sections with a duration of 5 s or less, and 10 non-overlapping data segments lasting 80 ms and centered at the spike peaks were manually selected to build power-spectra in each section of the ictal EEG. Spectral analysis was also undertaken by selecting 10 data segments at the time of spikes during an interictal period that was more than 1 min away from the seizure onset, and 10 segments at the time of spikes during a preictal period less than 60 s from the seizure onset. The length of the available interictal EEG data was 9.5 min in this recording. The minimal interval between the selected discharges was 130 ms.

The time–frequency spectral analysis was performed using the Gabor (Windowed Fourier) Transform (Kobayashi et al., 2004) with a sliding Gaussian window of 10 ms FWHM (full width half maximum). The frequency range was 100–1000 Hz. The Fourier transform was performed on 512 data points (51.2 ms; frequency resolution 19.5 Hz) at each time step, and the step was 1 ms. The signal power was converted to logarithmic scale to obtain a more Gaussian distribution (Gasser et al., 1982). The average spectra for each section were obtained by averaging the 10 spike-related data segments.

For statistical comparison of the power of fast activity between the discharges and the background, 1200 EEG segments lasting 51.2 ms and non-overlapping (61.4 s of EEG data in a total) were selected in the interictal background, and the Fourier transform was similarly applied to each background segment to obtain the control spectral data. The background segments were at least 1 s away from any spike. The unpaired *t*-test was performed between the discharge and control spectral data to obtain the *t* value at each pixel of the time–frequency spectrum (*t*-spectrum).

There is an enormous number of pixels in the time–frequency spectrum, and therefore we used a statistical procedure for controlling the false discovery rate (FDR) to avoid declaring too many pixels as active (type I errors) (Genovese et al., 2002): the details of statistical procedure are described by Kobayashi et al. (2009), and are briefly summarized. The FDR is defined as the ratio of the number of false positive pixels to the number of pixels declared

active, and one selects the FDR bound q that is the maximum tolerable FDR on average. In the present study, the two-tailed test was used with $q = 0.025$. Pixels with significantly great power are indicated in red, and pixels with t values that did not reach the limits are in green, in the FDR-controlled t -spectra (Fig. 2, full illustration in Supplementary Fig. 1). The computation was done with an in-house program written in MATLAB (version 6.5.1; MathWorks, USA).

We also temporally expanded the EEG discharges to visually investigate their detailed morphology after filtering with three different low-cut filters (bidirectional Butterworth, -6 dB) at 1.5, 80 and 250 Hz (Fig. 3, green, blue and red traces, respectively). The rates of occurrences of ripples and fast ripples per second were examined in each EEG section with respect to the right hippocampus, amygdala and parahippocampal gyrus: a ripple and a fast ripple were defined as an event of at least four consecutive oscillations with a frequency of 80–250 Hz and 250–500 Hz, respectively (Jacobs et al., 2008). The frequency, amplitude and duration of ripples and fast ripples were also visually measured. Temporal expansion of the EEG traces was also performed with three low-cut filters of 0.016, 80 and 250 Hz to show slow activity (Supplementary Fig. 2, green, blue and red traces, respectively). Note that the low-cut filter of 0.016 Hz means usage of no filter except for the filter before digital sampling (Ikeda et al., 1999).

Results

Increase of power of HFOs in association with the seizure discharges was indicated by the FDR-controlled time–frequency t -spectra. It was most profound in the hippocampal discharges, especially in the early part of seizure, when buildup of HFOs up to 800 Hz was observed (Fig. 2, RH1–RH2, ictal sections 1 and 2). In the hippocampal electrodes, increase of HFOs was noted below 500 Hz in association with the interictal spikes, and above 500 Hz in the preictal spikes heralding the greatest increase of HFOs at the seizure onset. Changes of HFOs were minimal with relatively low amplitude discharges in the amygdala (Fig. 2, RA1–RA2). Although the seizure discharges also involved the parahippocampal gyrus, increase of HFOs was largely below 500 Hz and less intense than in the hippocampus (Fig. 2, RB1–RB2, sections 2–4). The presence of HFOs with wavelength shorter than 2 ms (frequency of >500 Hz) in fast ripples was visually confirmed, especially in the hippocampal discharges during the preictal period and the initial part of seizure, by temporal expansion and filtration of the raw EEG traces (Fig. 3B–D). Appearance of the ictal slow activity coincided with the emergence of HFOs at the seizure onset (Supplementary Fig. 2C).

With respect to the changes of visually identified ripples and fast ripples according to the EEG sections, ripples and fast ripples were rare in the interictal and preictal periods. Most of the observed interictal high-frequency events had three oscillations or less (not countable as ripples or fast ripples) and lacked activity above 500 Hz (Fig. 3A). The rates of occurrences of ripples and fast ripples per second increased in association with the seizure, most markedly at the hippocampus: the increase in the rate of fast ripples at the initial part of seizure was more dramatic than the increase in the rate of ripples (Fig. 4A and B). The ratio of the number of fast ripples including activity above 500 Hz to that of all fast ripples at the hippocampus was low in the interictal period, began to increase from the preictal period, and

reached a peak at the seizure onset (Fig. 4C). The parameters of individual ripples and fast ripples (frequency, amplitude and duration) did not show any remarkable trend according to the EEG sections (Supplementary Fig. 3).

Discussion

HFOs in EEG of epileptic patients have been so far studied in the frequency range of up to 500 Hz. There is a technical limitation in the clinical EEG recording, though activity at 600 and 1000 Hz has been detected by averaging a large number of signals in somatosensory evoked potentials (SEP) (Eisen et al., 1984; Klostermann, 2005), and the occurrence of ultrafast activity has been indicated in animal experiment, as mentioned above (Rodin et al., 1973; Rodin, 2005). In the present report, we demonstrated the existence of HFOs above 500 Hz and up to 800 Hz in association with a seizure discharge in a patient with mesial temporal lobe epilepsy. This was demonstrated by statistical spectral analysis employing the FDR-controlled time–frequency t -spectra, and was also confirmed by the temporal expansion and filtration of the EEG traces.

The discharge-related buildup of HFOs above 500 Hz was most marked at the seizure onset but also started to occur in the preictal spikes. Assuming that the faster HFOs are, the more ictogenic they are, facilitation of ictogenesis might take place in association with the transition of neuronal system from the interictal to preictal state and to the initiation of the ictal state. We do not mean to define HFOs of any frequency as the initial ictal event, because HFOs and slower activity emerged almost simultaneously at the seizure onset (Supplementary Fig. 2C). In the present seizure originating from the hippocampus, HFOs above 500 Hz were observed in the hippocampus. It is still an open question whether HFOs above 500 Hz exist in seizures starting in other brain structures.

It is interesting to note that very high-frequency activity is usually considered to be generated in a locally confined neuron population (Bragin et al., 2002) and to be synchronized over small distances, and hence they would probably best be recorded with a microelectrode. We used electrodes with a surface of 3.6 mm² and it is remarkable that we could record these very high frequencies, implying that they must involve relatively large regions. Very high-frequency activity is suggested to spread via densely interconnected local neuron networks (Rampf and Stefan, 2006). HFOs with frequency above that of fast ripples may have even smaller primary generator network, and their detection by macro-electrodes during the preictal and beginning period of a seizure may reflect the spreading process of local epileptic activity with a possible igniting effect on the seizure.

The detection of such fast activity was possible by recording with a sampling frequency of 10 kHz. Note that it was a very rare chance to capture a seizure with this sampling frequency because our EEG system does not allow long-term recording with a large number of electrodes when the sampling frequency is this high. We do not believe that other clinical monitoring systems allow such recordings either. We have not yet succeeded to record more seizures with this sampling frequency. Although we report only one seizure, high-frequency activity above 500 Hz is unlikely to be rare given that this seizure was totally unselected. We are well aware that the current findings were substantiated in a single seizure and need future

confirmation. However such extended studies are possible only if a very high sampling frequency is intentionally used to record seizures, and otherwise important information about HFOs above 500 Hz would be missed. The present study is meant to encourage the future development of EEG systems that allow long-term recording with a full set of electrodes using a very high sampling frequency as well as the usage of such system despite large resources required. The EEG recording with a high sampling frequency will be beneficial for the elucidation of the pathophysiology of ictogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study is supported in part by the Japan Epilepsy Research Foundation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.eplepsyres.2009.10.008.

Figure 1.

The interictal and ictal EEG. (A) The interictal EEG trace recorded 8 min before the onset of seizure. Spikes are sporadically observed in the depth electrodes at the right hippocampus (RH1–RH2) and cortical electrodes at the right parahippocampal gyrus (RB1–RB2). (B) The preictal EEG trace recorded during the period within 60 s before the onset of seizure. Spikes are much more frequent in the preictal period than in the interictal period. (C) and (D) (continuous traces) The ictal rhythmic discharges start from RH1–RH2, and are also observed in RB1–RB2. The ictal EEG data were separated into sections with different morphologies and were analyzed as in Fig. 2. (E)–(G) Fusion images of CT and MRI showing the electrode locations (E, RA-depth electrodes targeting the right amygdala; F, RH-depth electrodes targeting the right hippocampus; G, RB-cortical electrodes on the basal part of the right temporal lobe). Arrows (a–d) correspond to the discharges in the expanded EEG traces (Fig. 3A–D, respectively).

Figure 2.

Time–frequency power-spectra and t -spectra controlled by false discovery rate (FDR) of EEG discharges. The temporally expanded and overlaid EEG traces of discharges and the corresponding power and FDR-controlled t -spectra are arranged in order from top to bottom, with respect to electrodes RA1–RA2 at the right amygdala in the top row, electrodes RH1–RH2 at the right hippocampus in the middle, and electrodes RB1–RB2 at the right parahippocampal gyrus in the bottom. The columns are arranged in order from left to right as the interictal and preictal periods and the initial part of the ictal sections (ictal sections 1–5, full illustrations in Supplementary Fig. 1) shown in Fig. 1. The frequency range is from 100 to 1000 Hz. Increase of power is indicated in red in the FDR-controlled t -spectra, and it is most remarkable in RH1–RH2 and minimal in RA1–RA2. Power of HFOs above 500 Hz increased especially in the beginning part of the seizure (ictal sections 1 and 2), and also in the preictal period. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Figure 3.

Temporally expanded and filtered traces of EEG discharges. The discharges (A–D) are expanded and correspond to arrows (a–d, respectively) in Fig. 1: they include an interictal spike (A), a preictal spike (B), and discharges in the beginning part of seizure (C and D). Three different low-cut filters (LCFs) at 1.5, 80 and 250 Hz (traces in green, blue and red, respectively) were applied to each discharge. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Figure 4.

Changes in ripples and fast ripples according to the EEG sections. The rate of occurrences of ripples (A) and fast ripples (B) per second is plotted according to the electrode locations and the EEG sections. Oscillations with three or less peaks were not counted. Fast ripples including visually identified activity above 500 Hz were observed only in EEG recorded from the hippocampus: the ratio of the number of fast ripples including activity above 500 Hz to the number of all fast ripples recorded from the hippocampus is also plotted (C).