High frequency oscillations and seizure frequency in patients with focal epilepsy

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Summary

High frequency oscillations (HFOs) have been associated with epileptogenicity. In rats, the extent of HFOs (>200 Hz) is correlated with seizure frequency. We studied whether the same applies to patients with focal epilepsy. Thirty-nine patients with intracerebral EEG sampled at 2000 Hz were studied for interictal ripples (80–250 Hz), fast ripples (FR, 250–500 Hz) and spikes. Seizure frequency before implantation was compared to numbers of channels with HFOs (>1/min). Analyses were repeated for HFO rates of >5, >10 and >20. Separate analyses were done for 25 patients with temporal lobe epilepsy only and for a selection of similar unilateral temporal channels in 12 patients. No linear correlation or trend was found relating the number of channels with HFOs and seizure frequency. There was a linear positive correlation between the number of channels with more than 20 FRs/min and seizure frequency. The hypothesis that the more tissue generating HFOs, the higher the seizure frequency, was not confirmed, though there might be a correlation for high FR rates.

Keywords

Intracranial electrodes; Epilepsy surgery; Ripple; Fast ripple; Seizure prediction

Introduction

High frequency oscillations (HFOs) have been detected in epileptic rats and in patients with epilepsy (Bragin et al., 1999, 2002; Fisher et al., 1992), with microelectrodes and clinical macroelectrodes (Jirsch et al., 2006; Khosravani et al., 2008; Worrell et al., 2008). They are divided into ripples (80–250 Hz) and fast ripples (FRs: 250–500 Hz) and partly co-occur with epileptic spikes (Jacobs et al., 2008; Urrestarazu et al., 2007). They have been associated with epileptogenesis and seizure genesis, as they are seen before the first seizure (in rats) and occur mostly in the seizure onset region and during seizure onset (Bragin et al., 2004; Jirsch et al., 2006).

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HFOs are seen ictally, and interictally mostly during slow wave sleep (Bagshaw et al., 2008). In kainic injected epileptic rats the spatial distribution of FRs was related to seizure frequency: a positive linear correlation between the number of electrodes showing FRs (>200 Hz) and seizure frequency was found (Bragin et al., 2003). This correlation resulted partly from two rats with bilateral HFOs having high seizure frequency, but the correlation remained after leaving these out of the analysis.

The latter finding suggests that the extent of FRs is related to disease severity in rats. Whether the same can be assumed for humans is unknown. In humans, the extent of higher frequencies during seizure onset is correlated to the duration of disease (Bartolomei et al., 2008). If HFOs are more widespread in patients with frequent seizures or long disease duration, they could offer a measure for disease severity, enabling a better estimation of potential outcome after treatment. The aim of this study is to see whether we obtain equivalent results in humans.

Methods

Patients

Thirty-nine of 42 patients with medically intractable focal epilepsy, who underwent intracranial depth stereo-EEG (SEEG) recorded at 2000 Hz (September 2004–September 2007), had EEGs that allowed the study of interictal HFOs (see below). The Montreal Neurological Institute and Hospital Research Ethics Committee approved the study and patients gave informed consent.

SEEG

During 10–21 days, patients were monitored with a 128-channel video-SEEG (Harmonie, Stellate, Montreal), at times acquired at 2000 Hz sampling rate after a 500 Hz low-pass hardware filter. Intracerebral electrodes (0.254 mm stainless steel core wrapped with 0.076 mm wire, coated with Teflon) were stripped focally yielding electrode contacts. The tip formed the deepest contact (1 mm length; effective surface 0.85 mm²) and eight other contacts were 5 mm apart (0.5 mm; 0.80 mm²). Electrode placement resulted from clinical decisions. To investigate the temporal lobe three depth electrodes were directed orthogonally through the middle temporal gyrus with the deepest contacts in the amygdala, anterior hippocampus and parahippocampal gyrus. Numbers and direction of extra-temporal electrodes varied. Electrodes were both in grey and white matter.

Marking HFOs and spikes

We selected the first night recorded at 2000 Hz with co-registered EMG and EOG channels. After spectral trend analysis of an EMG channel for muscle activity and a non-spiking depth EEG channel for delta activity, epochs with high delta and low EMG power were visually evaluated and 10 min of slow wave sleep (>25% delta) were selected at least 6 h away from any seizure.

HFOs were marked in bipolar montage using a vertically split screen (left: 80 Hz high-pass filter; right: 250 Hz high-pass filter). A ripple was marked if an event was clearly visible on

the side of the 80 Hz filter and did not occur or show the same shape on the side of the 250 Hz filter, as it is defined as a distinct event between 80 and 250 Hz in frequency. An event was regarded as a fast ripple if it was visible in the 250 Hz filter (supplementary figure). The time scale was set to maximal resolution (0.8 s/page on a 48 cm width monitor) and amplitude scale to 1 $\mu\text{V}/\text{mm}$ (supplementary figure). Spikes were reviewed on a separate EEG copy (10 s/page, 30 $\mu\text{V}/\text{mm}$). All channels, meaning those in grey and white matter, were analyzed, except non-functional channels or those located outside the brain. Because marking HFOs is very time consuming, the workload was split by randomly assigning patients to two observers (MZ, JJ). To minimize subjectivity, both observers marked 1 min for all patients and channels with low inter-observer agreement ($\kappa < 0.5$) were reviewed jointly to reach consensus on how to mark events. Then, the assigned reviewer marked 5 min accordingly. The stability of events over these 5 min was determined by analyzing the stability of event rates and the ranking of channels within these 5 min. The variance in event rates was estimated using a Jensen–Shannon divergence: if marking 5 min did not change the rate clearly compared to marking fewer minutes, the rate was considered stable and no information gain was expected from marking a longer epoch. Not only are the actual rates per minute important, but also the ranking of the channels containing HFOs and spikes. To determine how marking more minutes would affect the ranking of channels with respect to rates, a weighted version of the Damerau–Levenshtein (DL) distance was calculated. The DL distance measures the distance between two sequences (in our case rankings), in terms of the number of insertions, deletions, or transpositions required to render the two sequences identical. The cost associated with each operation was weighted based on the difference in rates of consecutive channels in the ranking. 10 min were marked if lack of stability in rate or localization of events could be expected from marking 5 min. This method was described previously in more detail (Zelmann et al., 2008).

Seizure frequency

The number of seizures during the 6 months preceding implantation was determined from the history included in the implantation EEG report. If this was not clear, the doctor's notes from the evaluation preceding the implantation were consulted. For some patients a seizure calendar was available. However, in most cases only a range was available. From this range, e.g. 2–4 per week, the average was taken. Seizure frequencies (per month) and the numbers of seizure days per month were calculated. Patients were considered to have secondarily generalized seizures if they experienced such seizures during the 6 months preceding implantation.

Analysis

A MATLAB program calculated for each patient the *number of channels* with more than one event per minute for ripples, FRs, spikes, ripples outside spikes and FRs outside spikes (HFOs can co-occur with spikes). These were correlated to seizure frequency and to the number of seizure days using linear correlation. Alternatively, a Mann–Whitney–Wilcoxon test was used to evaluate whether patients with low seizure frequency (up to and including median) had a lower number of channels with events than patient with high seizure frequency (above median). The *percentage of channels* with events out of the total number

of analyzed channels was also studied with correlation to compensate for different numbers of analyzed channels.

Analyses were repeated for >5, >10 and >20 events/min for only ripples and FRs to keep the number of comparisons low. Patients with partial epilepsy have inhomogeneous pathology and different electrode implantations. To decrease heterogeneity, analyses were repeated for patients with temporal epilepsy only. To decrease differences in electrode locations and numbers, analyses were repeated for electrodes limited to hippocampus, parahippocampus and amygdala in patients with unilateral mesiotemporal lobe epilepsy. Sometimes a channel was broken or outside the brain. Only channels marked in all patients were analyzed.

The number of channels with ripples and FRs (>1/min) was also correlated to the disease duration. We studied whether patients with secondarily generalized seizures had more channels with HFOs (>1/min; two-tailed *t*-test).

There might be more channels with events when more electrodes are implanted. This might bias results if seizure frequency is correlated to the number of implanted electrodes. This was studied by correlating seizure frequency with the number of implanted electrodes with linear correlation.

Results

For all 39 patients (mean age 36 years, 14 women) the average rate/min/channel was 13.7 ± 35 for ripples (mean rate of channel with highest rate per patient 137), 2.8 ± 22 for FRs (mean maximum rate 64) and 5.5 ± 11 for spikes (mean maximum rate 33). In five patients, 10 min instead of 5 min were marked because in 5 min rate or ranking was not stable. Twenty-four patients had temporal lobe epilepsy and were studied separately and 11 patients had unilateral mesiotemporal epilepsy and had 14 similar temporal electrode channels (averaging 40 channels) which could be compared (Table 1).

There was no correlation between seizure frequency and number or percentage of channels with ripples and FRs, nor with spikes, ripples outside spikes and FRs outside spikes (>1/min; Table 2). Fig. 1 shows the relation between ripples and seizure frequency and FRs and seizure frequency. Because of the distribution, a logarithmic scale was given for seizure frequency.

The Mann–Whitney–Wilcoxon test showed no significant difference between the number of channels with events (>1/min) in patients with low and high seizure frequency (median 10.8 seizures/month, *p*-values all >0.4).

Analyses were repeated for higher HFO rates. The number of channels with more than 20 FRs/min was positively correlated with seizure frequency for the group of all patients and for the group with temporal lobe epilepsy (Table 2, Fig. 1). The analyses of 14 similar channels in 11 patients with unilateral mesiotemporal lobe epilepsy showed trends towards negative correlations (Table 2).

There was no significant correlation between disease duration and ripples (-0.09) or FRs (-0.32). Patients with secondarily generalized seizures did not have more channels with HFOs than patient with partial seizures only (ripples: 16.8 vs. 15.5 $p = 0.53$; FRs: 4.3 vs. 4.3 $p = 0.97$).

The number of analyzed channels was positively linearly correlated with the seizure frequency ($\rho: 0.48$; $p < 0.01$) and with the number of channels with ripples ($\rho: 0.48$; $p < 0.01$), while not with FRs ($\rho: 0.17$; $p = 0.29$).

Discussion

This study tested the hypothesis that the more tissue shows HFOs, the higher the disease load in terms of seizure frequency in patients with focal epilepsy. The basic comparisons in 39 patients did not find a positive linear correlation. There is however a positive correlation when considering channels with high FR rates.

Great disease heterogeneity in patients could explain the negative results. The epileptogenicity of areas with similar extent of HFOs may differ with underlying pathology. The seizure frequency was on average higher for dysplasias, so it would be interesting to study the different pathologies separately. Patient numbers were however too low to study correlations within separate pathologies. Another explanation could be differences between brain structures, especially between mesiotemporal and other structures. Separate analyses of patients with temporal lobe epilepsy did not alter conclusions, however. For unilateral mesiotemporal lobe epilepsy there might even be trends towards negative correlations, especially for spikes. This may result from chance, but implies that a positive correlation is unlikely. The negative results might also be related to a sampling error: electrodes were placed for clinical purposes according to standard procedures, for instance often in the hippocampus, amygdala and parahippocampus. A higher sampling rate with more electrodes and in additional areas like the temporopolar and entorhinal cortices might have yielded different results.

In rats, a positive correlation has been found between the number of channels with fast ripples and the seizure frequency (Bragin et al., 2003). The number of channels with high FR rates ($>20/\text{min}$) was positively correlated with seizure frequency, though results were not corrected for multiple comparisons. It is possible that only channels with relatively high rates of FRs are related to seizure load in humans, rather than channels with sporadic HFOs. Sporadic HFOs might be related to spikes and have different underlying pathophysiology. Ripples might partly represent physiological events and might even decrease in epileptogenic tissue (Staba et al., 2007). They were therefore not taken into account in the study of rats. It might be that including ripples in our analysis has diluted the results. Also, the rat studies were in hippocampal and parahippocampal structures, which were recorded from in only some of our patients. Another difference between our study and the study in rats is the number and size of the electrodes used. The use of bigger and fewer electrodes might result in relatively few channels with HFOs because pathological HFOs exist in relatively small, discrete islands of neurons. Indeed the number of channels with fast ripples was in general relatively low.

An interesting finding of this study is that patients with high seizure frequency have more electrodes implanted than patients with lower seizure frequency. Apparently patients with worse disease severity have more regions that were thought to be possibly clinically relevant, which in turn might have to do with suspected secondary epileptogenesis. Another explanation might be that in patients with a lot of seizures, the threshold for implantation is lower, even if the expected benefit is relatively low.

The influence of medication and therapy compliance are confounds and seizures might be under-reported (Davis et al., 2008; Hoppe et al., 2007). These factors are difficult to control. Also, the occurrence of HFOs might be influenced by medication dose. This suggests that it might have been fairer to evaluate seizure frequency during implantation, similarly to the rat study. However, seizure frequency during implantation is very dependent on changes in medication dose during the whole implantation period, which differs between patients. Therefore the seizure frequency before implantation seems more reliable and was evaluated instead of the number of seizures during implantation. For each patient the first night recorded at 2000 Hz was selected. Due to practical reasons this was not always the same night and different selections were at different levels of anti-epileptic drug withdrawal. However, a previous study showed that, although the rate of HFOs changes with altered medication dose, the number of channels with HFOs does not (Zijlmans et al., 2009). There was another potential bias: patients with more seizures had more electrodes and patients with more electrodes had more channels with ripples, which could have led to false positive correlations. Therefore, percentage rather than absolute number of channels, and a subset of channels in a subset of patients were analyzed, but this did not alter conclusions.

Future studies should determine which HFOs are pathological. If disease load can be estimated from the spatial extent of HFOs, this would provide a clinical tool for optimizing therapy. Also, it would be interesting to see whether the extent of HFOs relates to postsurgical outcome. It would be interesting to repeat the study in different patients, maybe with grid electrodes, to see whether the results can be replicated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

The relation between seizure frequency per month and number of channels with (A) ripples (>1/min), (B) fast ripples (>1/min), and (C) more than 20 fast ripples per minute. There were no patients with 0 channels with ripples (>1/min; A), but there were patients with 0 channels with fast ripples (>1 or >20/min; B and C). The seizure frequency was shown on a logarithmic scale, because of the distribution. As indicated in the text, there was no correlation between seizure frequency per month and the number of channels with more than 1 ripple or fast ripple per minute, but there was a positive correlation between seizure frequency and more than 20 fast ripples per minute.

Table 1

Patient characteristics for all patients, for patients with temporal lobe epilepsy and for patients with unilateral mesiotemporal lobe epilepsy with electrodes in the amygdala, hippocampus and parahippocampus.

Patients	All (39)	Temporal (24)	Unilateral mesiotemporal (11)
M:F ratio	25:14	14:10	5:6
Age (years)	36 ± 11	35 ± 12	36 ± 14
Disease duration (years)	22	19	17
Seizure frequency (<i>n</i> /month)	40 ± 107	51 ± 135	36 ± 68
Range seizure frequency	0.4–645	0.4–645	0.4–645
Seizure-days/month	15 ± 12	14 ± 12	13 ± 12
# patients with sec generalized seizures	19	13	4
Mean # channels studied	39	42	14 out of 40
MRI abnormalities (<i>n</i>)	28	17	6
MTS or MT atrophy (<i>n</i>)	8	8	2
Previous resection (<i>n</i>)	7	4	0
Dysplasia (<i>n</i>)	8	3	2

Sec = secondarily. MTS = mesiotemporal sclerosis. MT = mesiotemporal.

Table 2

This table shows the correlation coefficients Rho for different alternative comparisons: seizure frequency (seizures/month) compared to the number and percentage of channels with ripples, fast ripples, spikes and ripples without spikes (first two lines), seizure frequency compared to number of channels with higher rates of ripples and fast ripples (>5, >10 and >20, lines 3–5) and number of seizure-days/month compared to channels with ripples and fast ripples. All comparisons were done for all patients, all patients with temporal lobe epilepsy and all patients with unilateral mesiotemporal seizure onset.

Rho	Ripples			FR			Spikes			R isol			FR isol		
	All	T	UT	All	T	UT	All	T	UT	All	T	UT	All	T	UT
Seizures/month															
# channels >1/min	0.20	0.26	-0.30	-0.04	-0.16	-0.37	0.02	-0.08	-0.55	0.22	0.35	-0.17	0.02	-0.07	-0.34
% channels >1/min	-0.12	-0.23	n/a	-0.18	-0.08	n/a	-0.18	-0.42	n/a	-0.10	-0.18	n/a	-0.15	-0.28	n/a
# channels >5/min	0.14	0.18	-0.44	0.10	0.03	-0.49									
# channels >10/min	0.14	0.19	-0.20	0.26	0.25	-0.43									
# channels >20/min	0.14	0.19	-0.31	0.44 *	0.45 *	-0.30									
Seizure-days/month															
# channels >1/min	0.05	0.10	-0.26	-0.13	-0.08	-0.43									
% channels >1/min	-0.11	-0.20	n/a	-0.30	-0.32	n/a									

FR: fast ripples, R isol: ripples outside of spikes, FR isol: fast ripples outside of spikes, All: all 39 patients, T: 24 patients with temporal lobe epilepsy, UT: comparison of 14 similar channels in 11 patients with unilateral mesiotemporal epilepsy. Correlation coefficients for spikes, isolated ripples and isolated fast ripples were only calculated for channels with rates $\geq 1/\text{min}$.

* $p < 0.05$.