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High-frequency oscillations and seizure generation in neocortical epilepsy

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Summary

Neocortical seizures are often poorly localized, explosive and widespread at onset, making them poorly amenable to epilepsy surgery in the absence of associated focal brain lesions. We describe, for the first time in an unselected group of patients with neocortical epilepsy, the finding that high-frequency (60–100 Hz) epileptiform oscillations are highly localized in the seizure onset zone, both before and temporally removed from seizure onset. These findings were observed in all six patients with neocortical epilepsy out of 23 consecutive patients implanted with intracranial electrodes for pre-surgical evaluation during the study period. The majority of seizures (62%) in these patients were anticipated by an increase in high-frequency activity in the 20 min prior to neocortical seizure onset. Contrary to observations in normal brain, high-frequency activity was strongly modulated by behavioural state, and was maximal during slow-wave sleep, which may explain the propensity for neocortical onset seizures to begin during sleep. These findings point to an important role for neuromodulatory circuits, probably involving the thalamus, in mechanisms underlying seizure generation in neocortical epilepsy. These findings demonstrate that high-frequency epileptiform oscillations may prove clinically useful in localizing the seizure onset zone in neocortical epilepsy, for identifying periods of increased probability of seizure onset, and in elucidating mechanisms underlying neocortical ictogenesis. Confirmation that prolonged bursts of high-frequency activity may predict focal onset neocortical seizures will require prospective validation on continuous, prolonged recordings in a larger number of patients. Importantly, the results show that the dynamic range utilized in current clinical practice for localization of epileptogenic brain largely ignores fundamental oscillations that are signatures of an epileptogenic brain. It may prove that many currently available clinical EEG systems and EEG analysis methods utilize a dynamic range that discards clinically important information.

Keywords: EEG; epilepsy; high-frequency oscillations; ictogenesis; sleep

Abbreviations: HFEO = high-frequency epileptiform oscillation

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Introduction

The range of EEG oscillation frequencies thought to be physiologically important for human brain function extends well beyond the frequency bandwidth originally considered by its discoverer, Hans Berger (Berger, 1929; Curio, 2000*a*). Recent studies describe gamma oscillations (~30–60 Hz) that are believed to play a role in learning and memory (Lisman and Idiart, 1995; Llinas, 1988), ripple oscillations (~100–200 Hz) that may be important for memory consolidation (Buzsaki, 1989, 1996; Bragin *et al.*, 1999*a*; Grenier *et al.*, 2001), and evoked potential oscillations (~600 Hz) (Curio, 2000*b*) produced by somatosensory stimulation. In addition to normal brain function, high-frequency oscillations (>60 Hz) have been described in human and animal epileptic foci (Allen *et al.*, 1992; Fisher *et al.*, 1992; Alarcon *et al.*, 1995; Bragin *et al.*, 1999*b*; Traub *et al.*, 2001; Spencer and Lee, 2000; Grenier *et al.*, 2003).

Nonetheless, the majority of human studies using intracranial EEG, performed as part of pre-surgical evaluation in

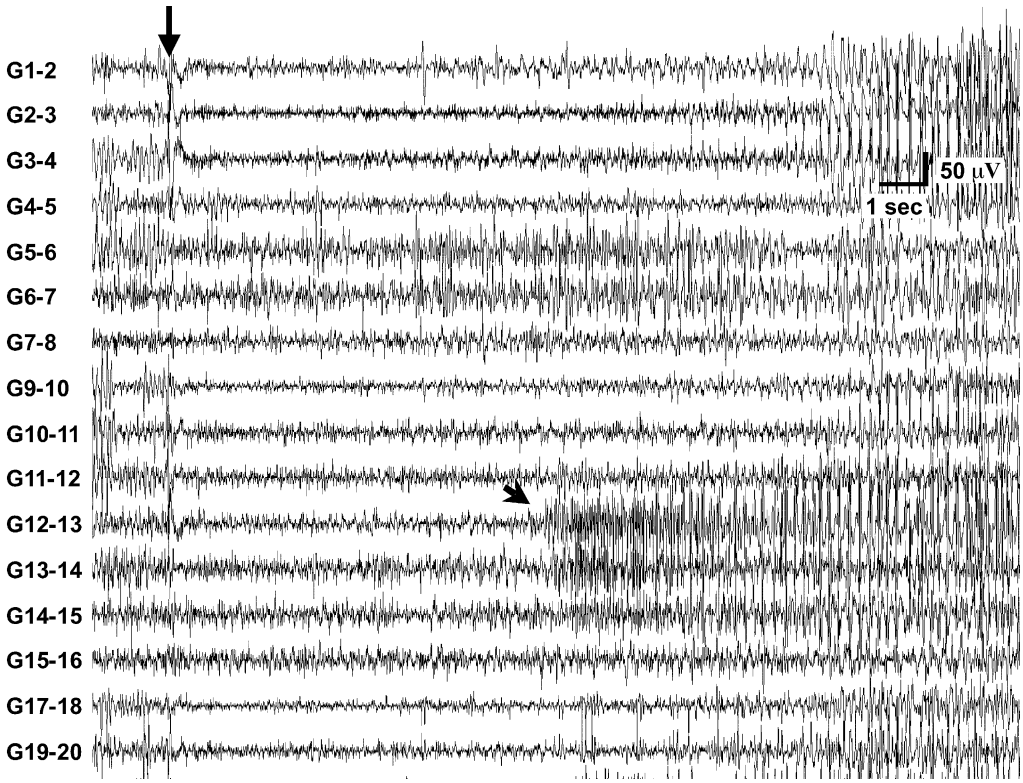

Fig. 1 Seizure onset (arrow) in patient 3. There is 94 Hz synchronous activity that is most prominent at G2–3 (small arrow). (See Fig. 2 for an expanded view of the seizure onset.) This discharge spreads to involve the adjacent contacts G11–12 (large arrow) 6 s later.

patients with intractable epilepsy, usually report a limited frequency range (~0.1–30 Hz) of EEG activity (Quesney *et al.*, 1992; Schiller *et al.*, 1998; Quesney, 2000). This limited frequency range may reflect the fact that oscillations with frequencies above ~30 Hz are relatively low amplitude and are obscured by lower frequency activity (Nunez, 1981). For this reason, and the fact that most commercial EEG systems employ low-pass filters with cut-off frequencies from 70 to 100 Hz, we suggest that high-frequency oscillations are probably under-recognized in human intracranial EEG studies.

In fact, recent studies in selected groups of patients show that neocortical seizures can begin with low-amplitude high-frequency oscillations (Allen *et al.*, 1992; Fisher *et al.*, 1992; Alarcon *et al.*, 1995; Traub *et al.*, 2001); and in the interictal (between seizures) period there are high-frequency epileptiform oscillations (HFEOs) present (Bragin *et al.*, 1999*a*,*b*; Traub *et al.*, 2001), suggesting that HFEOs may be involved in seizure generation (ictogenesis) (Traub *et al.*, 2001). Previous studies, however, report on highly selected patients, and the actual prevalence of HFEO in neocortical epilepsy remains unknown. Whether interictal HFEOs can spatially localize epileptogenic brain, and the role of HFEOs in seizure generation are unclear. For these reasons, we investigated patients with neocortical onset seizures undergoing monitoring with subdural–intracranial electrodes as part of their evaluation for possible epilepsy surgery. In contrast to mesial temporal lobe onset seizures, neocortical onset seizures are often poorly localized, explosive and widespread at onset, making individuals with these seizures poor candidates for epilepsy surgery. We hypothesized that HFEOs might be useful in localizing the functional seizure onset zone in these difficult to treat patients with neocortical epilepsy, which is critical for successful epilepsy surgery, and for identifying periods of increased probability of seizure activity.

The presence of a reliable seizure precursor, a harbinger of focal neocortical seizures, would open a therapeutic window possibly allowing interruption of ictogenesis and preventing seizures (Litt *et al.*, 2001; Litt and Lehnertz, 2002). Better localization of the seizure onset zone and epileptogenic brain by mapping HFEOs in neocortical epilepsy might lead to better techniques for surgically disrupting the epileptic network in these patients.

Methods

Subjects

Twenty-three consecutive patients who underwent long-term continuous video and intracranial EEG monitoring at Emory University and University of Pennsylvania as part of their evaluation for epilepsy surgery were studied. Each patient gave their informed consent for participation in these research studies under approval of the Emory University and University of Pennsylvania Internal Review Boards. Two clinical epileptologists (B.L. and G.A.W.)

reviewed and marked each patient's entire video-EEG record for seizures (Fig. 1) and behavioural state.

EEG electrodes

Each patient had intracranial electrodes placed according to standard pre-surgical evaluation protocols (Engel, 1987; Quesney *et al.*, 1992; Quesney, 2000). Ad-Tech subdural grids, strips and depth electrodes (AD-TECH Medical Instrument Corporation, Racine, WI) were used for all studies. Subdural electrodes had varying numbers of contacts, depending on the clinical requirements. The individual electrode contacts of the subdural grids are 4.0 mm diameter platinum discs with a centre-to-centre electrode distance of 10 mm. Patients requiring mesial temporal recordings had 8-contact depth electrodes stereotaxically placed into each temporal lobe via occipital or temporal burr holes.

Intracranial EEG data collection and storage

Continuous video and intracranial EEG were collected using a digital, 64-channel, 12-bit, Nicolet BMS-5000 (Nicolet Biomedical, Madison, WI) epilepsy monitoring system, and stored on videotape. Referentially recorded EEG was band-pass filtered from 0.1 to 100 Hz, digitized at 200 Hz and archived to compact disc (CD-ROM) for later processing. Bipolar electrode montages were used to eliminate common mode artefact, and a digital 60 Hz notch filter was employed to eliminate line noise. Data were processed using custom MATLAB (Mathworks, Natick, MA) programs. Hospital stays varied from 3 to 27 days, yielding ~1.5 Gbytes/day of raw EEG data.

Behavioural state

Videotapes were viewed in their entirety for clinical seizures, and approximation of sleep/wake periods. Finer resolution of sleep/wake state was achieved by examining simultaneous EEGs to identify periods of slow-wave sleep characterized by widely distributed delta frequency activity (0.1–4 Hz). Because the clinical recording montage did not include electrodes for recording eye movements or muscle activity, complete sleep staging (Rechtschaffen and Kales, 1968) was not possible. Therefore, we characterized the entire hospital stay into four possible behavioural states: wakefulness; slow-wave sleep; seizure; or indeterminate. Only slow-wave sleep, wake and seizure records were selected for further analysis.

Determination of seizures, seizure onset zone and interictal epileptiform activity

Seizure onset times were determined by visual identification of a clear electrographic seizure discharge, and then looking backwards in the record for the earliest EEG change from baseline associated with the seizure. The earliest EEG change was selected as the seizure onset time. The seizure onset zone, defined by the electrode(s) with the earliest onset of seizure activity, was documented for each seizure, and classified as mesial temporal, neocortical temporal or neocortical extra-temporal. In patients with independent mesial temporal and neocortical onset seizures, only the neocortical onset seizures were selected for analysis. The electrode contacts exhibiting interictal spikes, sharp waves (Engel, 1987) and/or HFEOs (>60 Hz) were identified by visual review (Fig. 3). Lastly, electrodes far removed from the seizure onset zone were chosen as normal/control/non-epileptogenic brain. If available, the homologous region contralateral to the seizure onset zone was selected as the normal control for that patient.

Data analysis

Neocortical seizures were analysed provided the seizure onset was preceded by at least 2 h of uninterrupted artefact-free interictal EEG. Under these strict criteria for inclusion, only two neocortical seizures were excluded, except in patient 21 who had multiple seizure clusters. In this case, only the initial seizure of each cluster was analysed. For each patient's analysis, EEG seizure records (2 h records containing the seizure onset at 1 h and 50 min), and 10 each of randomly selected 15 min slow-wave sleep and wake baseline records were analysed. Seizure onset times and the seizure onset zone, both determined by visual review, were confirmed by time–frequency analysis (Figs 1 and 2). The frequency of oscillation at seizure onset was then determined by spectral analysis, selecting the frequency of the dominant spectral peak obtained from the initial 3–5 s at seizure onset (Fig. 2). The spatial location and frequency of interictal HFEOs were identified visually and confirmed using time–frequency and spectral analysis (Fig. 3).

In addition to HFEOs, the level of broadband high-frequency activity was quantified by band-pass filtering (60–100 Hz) raw EEG with a 6-pole Butterworth filter and then creating an average energy time series for each channel defined by:

$$E[k] = \frac{1}{M} \sum_{n=1+(k-1)(M-D)}^{k(M-D)+D} V^2[n] \quad \text{for } k = 1, 2, \ldots \tag{1}$$

where $V[n]$ is the bipolar voltage between adjacent contacts at discrete time t_n, M a 0.100 second window (M = 20 data points), and D a 0.045 second window overlap (D = nine points). Average high-frequency energy time series were created for seizure records, and the 150 min of randomly selected interictal slow-wave sleep and wake records.

Statistical analysis

A large degree of variability in the high-frequency energies was observed between patients due to the wide range of effective impedance values for subdural electrodes. This was the case despite attempts to optimize electrode impedance and to minimize artefacts. For this reason, we performed statistical analysis on each patient individually rather than across all patients. For each patient, all the EEG time series, 10 randomly selected 15 min EEG baseline records from slow-wave sleep and wake, and the 120 min seizure records were first converted to a running average energy time series (1) for each electrode site. In order to best probe for statistically significant differences in high-frequency energy at different electrode sites and/or during different behavioural states, we first obtain a normal distribution of the average energy data using the log transform of the high-frequency energy (Gasser *et al.*, 1982). The spatial distribution of broadband high-frequency activity in the seizure onset zone and normal brain regions in both wake and sleep periods was investigated using analysis of variance (ANOVA). For *post hoc* analysis, Tukey's multiple comparison tests was used. For each patient, the 'within-group' measures were repeated high-frequency energies obtained from 10 randomly selected 15 minute records. The 'between-group' measures included the seizure onset zone

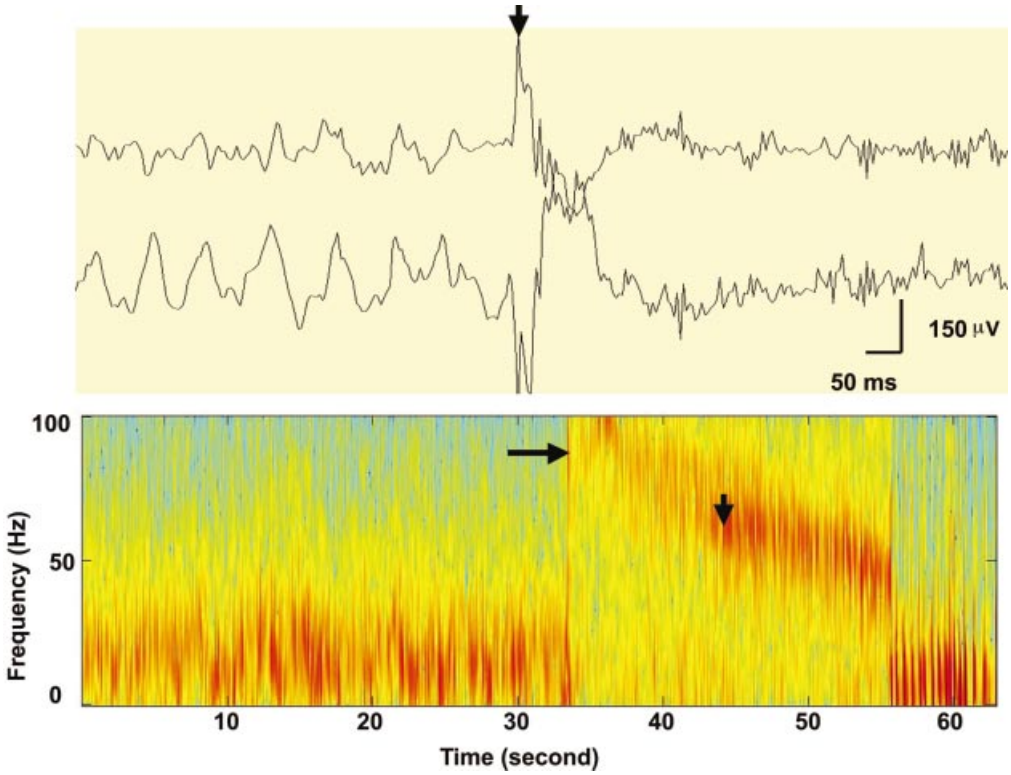

Fig. 2 The two upper traces are channels G2–3 and G3–4 from Fig. 1. The seizure onset (arrow) is reproduced on a scale where the synchronous high-frequency activity is apparent. The lower figure (different time scale) is a time–frequency plot showing the dominant spectral activity (red and indicated by the horizontal arrow) in the 90–100 Hz range at seizure onset (time of seizure onset is indicated by the vertical arrow). The arrowhead in the lower figure indicates the time of clinical onset (right arm clonic movements).

(epileptogenic brain), normal brain, and sleep and wake behavioural states for each brain region. The F statistic is used to test the null hypothesis that there is no difference in the high-frequency energy between behavioural states, wake and slow-wave sleep, in the epileptogenic and control/normal/non-epileptogenic brain for a given subject.

To determine if the high-frequency energy is increased within epileptogenic brain in the time period prior to seizure onset, we investigate the null hypothesis that the average high-frequency energy within the seizure onset zone is unchanged in the 20 min period prior to seizure onset. The hypothesis is tested by creating random samples of high-frequency energies in the pre-seizure period and in the interictal period (temporally far removed from seizures and the same behavioural state). A 20 min period is required to allow sufficient statistical power, i.e. to obtain enough samples of high-frequency energy to test the hypothesis. If the time period is significantly shortened under 20 min, in an effort to obtain greater temporal resolution, the statistical power needed to refute the null hypothesis is lost. A pre-seizure period, significantly different from the interictal period, was identified when the t test demonstrated that the null hypothesis could be rejected with $P < 0.05$ confidence.

Results

After the entire video and intracranial EEG of each patient was reviewed, six (26%) patients from the 23 consecutive patients studied had either exclusively neocortical, or neocortical and mesial temporal lobe seizures. The remaining 17 patients had exclusively mesial temporal lobe onset seizures. Of the six patients with neocortical onset seizures, three had seizures strictly from the dorsal lateral frontal convexity, and the remaining three had mesial temporal lobe and either neocortical temporal or frontal lobe onset seizures (Table 1). A total of 34 neocortical onset seizures met the inclusion criteria for analysis. In all the seizures analysed, a single (contiguous electrodes) neocortical seizure onset zone was identified, except for patient 7 who had independent frontal and temporal neocortical seizures.

Seizures and seizure onset zone

As described above, seizure onset times, seizure onset zone location and frequency of the dominant seizure onset oscillation were determined (Table 1). Patient 7 had an independent frontal lobe neocortical focus and temporal lobe neocortical focus [denoted in Table 1 as Pt 7(1) and Pt 7(2), respectively]. The remaining five patients had a single seizure onset zone focus from which all neocortical seizures were initiated. In each patient, the seizure onset zone spanned between three and five contiguous electrodes, except patient 21 who had a large seizure onset zone involving seven

Table 1 *Seizure characteristics*

Patientt	Seizure onset zone (no. of seizures)	Seizure onset frequency (Hz)	High-frequency activity (Hz)	Pre-seizure HFEO seizure number (% change HFEO)
2	Temporal (5)	24.4 ± 2.5 (55.6)*	73.0 ± 7.6 (50.7)*	1. 1.49^{+} 2. 0.78^{+} 3. 0.05 4. No change 5. 0.38^{+}
3	Frontal (7)	94.0 ± 3.7	84.1 ± 7.6	1. 0.61^{+} 2. No change 3. 4.13^{+} 4. 2.26^{+} 5. 3.3^{+} 6. 0.05 7. 5.98^{+}
7†	Temporal (3) Frontal (1)	21.9 ± 2.3 (65.1)* 53.1	62.4 ± 1.9 (54.8)*	1. 0.36* 2. 0.41^{+} 3. 0.13 4. 0.05
9†	Frontal (6)	73.4 ± 7.4	70.31 ± 5.8	1. 0.39^{+} 2. 0.36^{+} 3. 0.15 4. 0.37^{+} 5. No change 6. No change
20	Frontal (4)	67.1 ± 4.1	77.7 ± 9.5	1. 0.25^{+} 2. 1.28^{+} 3. 0.16^{+} 4. 0.24^{+}
21	Frontal (8)	66.1 ± 7.9	68.0 ± 4.2	1. 0.89^{+} 2. 0.15 3. 0.15 4. 0.14 5. 0.29^{+} 6. 2.5^{+} 7. 5.8^{+} 8. No change
	Total seizures			21 (62%) of seizures had a significant increase in HFEOs
	Temporal (7) Frontal (23)	23.7 ± 2.8‡ 74.3 ± 13.7¶	68.3 ± 4.2‡ 76.6 ± 9.8¶	

*Denotes frequency of a second prominent spectral power peak; $^{+}$statistically significant ($P < 0.05$) change in the high-frequency activity in the pre-seizure period; †patients had mesial temporal lobe onset seizures that are not included in the analysis; ‡dominant frequency of seizure onset [significant difference between frontal and temporal ($P < 0.05$)]; ¶dominant interictal HEFOs [significant difference between frontal and temporal ($P < 0.05$)].

contacts. All 34 neocortical seizures had prominent high-frequency spectral components (60–100 Hz) at seizure onset, which were the dominant spectral peaks in frontal lobe onset foci. In neocortical temporal lobe onset seizures, there were prominent high-frequency spectral components present, but the dominant onset oscillation was at a lower gamma frequency (Table 1).

Interictal discharges and behavioural state

HFEOs were found to be localized to only the seizure onset zone in all the patients studied. The frequency characteristics of interictal HFEOs are shown in Table 1. In all patients, the pathological HFEOs were stereotypic and clearly distinguishable from normal background activity (e.g. Fig. 3). HFEOs were brief in duration (average duration = 75 ± 33 ms, n = 100) with a low relative amplitude (average amplitude = 65 ± 40 μV, n = 100) compared with oscillations in the 1–30 Hz frequency band. The HFEOs were intermittently present throughout the interictal record, and often occurred asynchronously at different electrodes within the seizure onset zone (Fig. 3). In addition to these prominent interictal HFEOs, there was also an increase in the broadband (60–100 Hz) high-frequency activity in the seizure onset zone compared with control/normal/non-epileptogenic brain, which was apparent even during records that did not contain

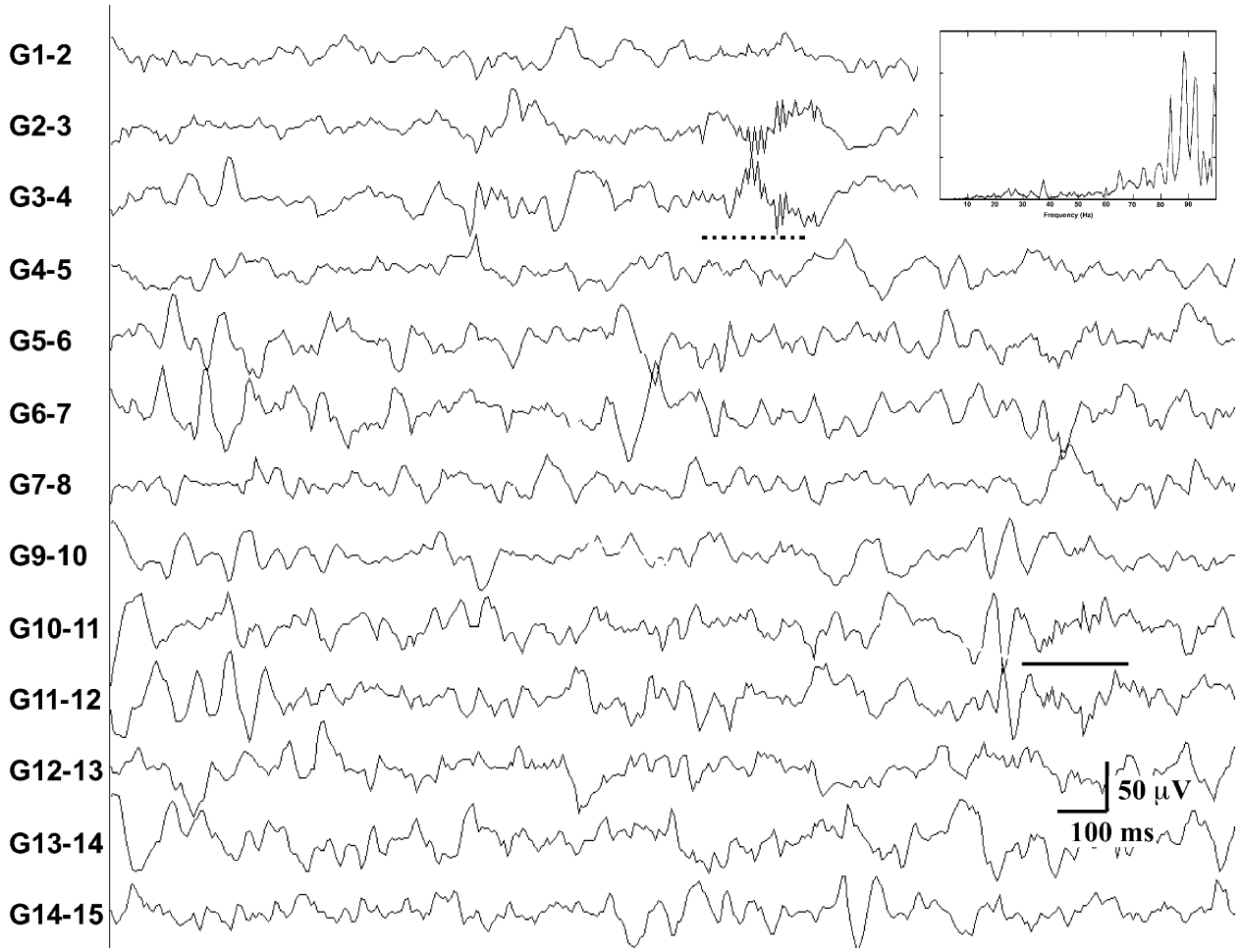

Fig. 3 Interictal HFEOs within the seizure onset zone (G2–3 and G3–4 are adjacent contacts with G10–11, G11–12). The inset panel shows the high-frequency spectral peaks that make up the oscillations. The interictal HFEOs have similar spectral characteristics to the seizure onset oscillations. Note that the interictal HFEOs occur simultaneously at the different contiguous sites.

clear discrete HFEOs. The increase in broadband activity was quantified by comparing the average energy of filtered (60–100 Hz) EEG in the seizure onset zone and control/normal/non-epileptogenic brain regions. In Fig. 4, the average high-frequency energies from epileptogenic (seizure onset zone) and non-epileptogenic/normal/control brain are shown. The energy in epileptogenic brain was greater than in the normal control region in all patients, and significant at the level $P < 0.05$ in all seizure onset zone foci, except for the temporal neocortical focus of patient 7(2) [the F statistics are $F_{\text{pt }2}(3,150) = 6.48$, $F_{\text{pt }3}(3,150) = 6.87$, $F_{\text{pt }7(1)}(5,150) = 4.31$, $F_{\text{pt }7(2)}(5,150) = 2.25$, $F_{\text{pt }9}(3,150) = 3.86$, $F_{\text{pt }20}(3,150) = 6.412$, $F_{\text{pt }21}(3,150) = 3.89$].

Within the seizure onset zone of all six patients there was increased high-frequency energy and HFEO activity during slow-wave sleep compared with wakefulness, which reached statistical significance in three of six patients [$F_{\text{pt }2}(3,150) = 2.77$, $F_{\text{pt }3}(3,150) = 3.51$, $F_{\text{pt }7(1)}(5,150) = 0.56$, $F_{\text{pt }7(2)}(5,150) = 0.15$, $F_{\text{pt }9}(3,150) = 3.31$, $F_{\text{pt }20}(3,150) = 0.08$, $F_{\text{pt }21}(3,150) = 14.3$]. Outside the seizure onset zone, in

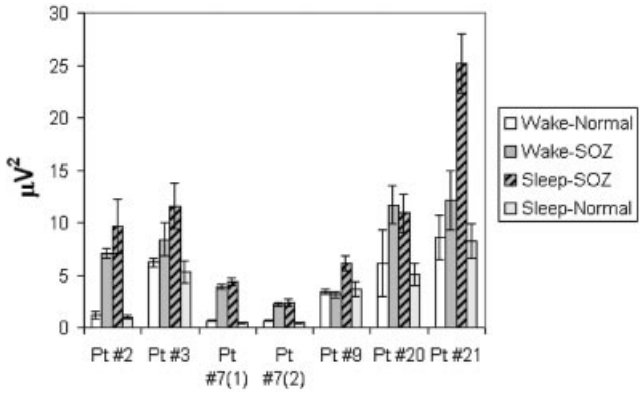

Fig. 4 Interictal broadband high-frequency (60–100 Hz) activity in the seizure onset zone (SOZ) compared with non-epileptogenic/normal/control brain. The figure shows a significant increase in high-frequency activity in the seizure onset zone that is modulated by behavioural state. No significant difference between sleep and wake states was found in the non-epileptogenic/normal/control brain.

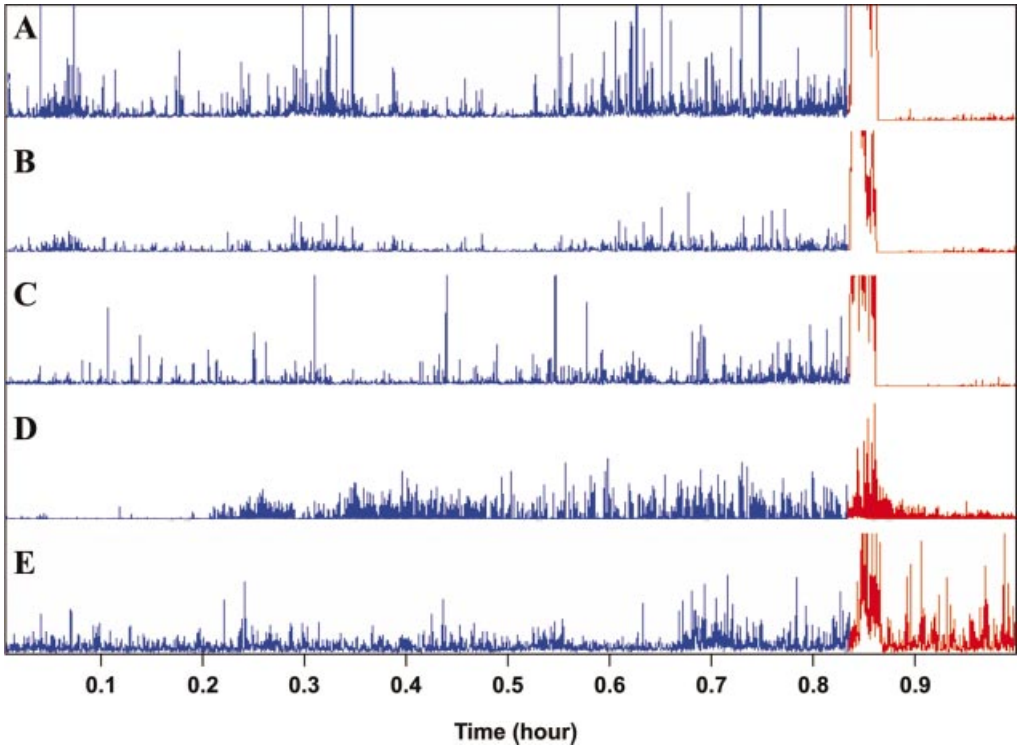

Fig. 5 Examples of the increase in high-frequency energy within the seizure onset zone prior to seizure onset (onset in red). The figure shows the high-frequency energy for 50 min (blue) prior to seizure onset (red). The majority of seizures studied showed increased high-frequency epileptiform energy prior to seizure onset when compared with baseline energies from EEG epochs that are temporally removed from the seizure onsets.

normal control brain, there was no significant difference in broadband high-frequency activity between sleep and wakefulness. In addition to HFEOs, there were epileptiform spikes and sharp waves identified in all patients. In all the patients studied, HFEOs and the region of increased (sleep and wake) broadband high-frequency activity were localized to the seizure onset zone, whereas epileptiform spikes and sharp waves had a much wider distribution involving regions outside the seizure onset zone. As this analysis was performed retrospectively, after patients underwent resective surgery based upon the results of standard intracranial video-EEG monitoring, the data required to determine if resection of the interictal HFEO zone is associated with excellent surgical outcome is limited in our study. Unfortunately, only in patients 20 and 21 was detailed information available regarding which electrode sites were actually resected. In the remaining four patients, the exact extent of surgical resection relative to the positions of the intracranial electrodes was not clear, and correlation of surgery outcome with degree of resection of the epileptogenic brain defined by interictal HFEOs is not possible. In the two patients where the information was available, we found that resection of electrodes exhibiting HFEOs was predictive of excellent post-surgical outcome (patient 20 is seizure free 1 year after surgery that removed the epileptogenic zone defined by ictal and interictal HFEOs. Patient 21 continues with frequent seizures after surgery that did not remove the seizure onset zone determined by ictal and interictal HFEOs).

Pre-seizure high-frequency activity

To investigate whether seizures are preceded by precursors of increased high-frequency activity and HFEOs, we examined the discrete average high-frequency energy time series in the period prior to seizure onset within the seizure onset zone. The time series consists of sequential high-frequency energies obtained as defined in equation (1). The average high-frequency energy time series provides multiple measurements of high-frequency neuronal activity. To compare the amount of high-frequency activity in the pre-seizure period (just prior to seizure onset) with times distant from seizure activity, we use the Student *t* test. The statistics of multiple measures of high-frequency activity in the seizure onset zone during the pre-seizure period (defined for this study as 20 min prior to the onset of a seizure on the EEG) were compared with randomly selected 15 min records (with the same behavioural state) that were temporally far removed from the seizure onset time (>2 h from any seizures). An identifiable pre-seizure period was considered to be present if the average high-frequency energy in the pre-seizure period was significantly different ($P < 0.05$) from the average high-frequency energy in the randomly selected samples.

In 21 (62%) of the 34 neocortical seizures analysed, there was a statistically significant increase in the broadband high-frequency activity within the seizure onset zone in the 20 min period prior to the seizure onset (Fig. 5 and Table 1). The increase in average high-frequency activity prior to seizure onset appears to be due to both broadband asynchronous activity and HFEOs. This result supports the concept that high-frequency activity and HFEOs within the seizure onset zone may serve as seizure precursors, useful for identifying periods of increased probability of clinical seizures.

Discussion

In all six (100%) patients with neocortical seizures, the seizure onset on intracranial EEG demonstrated significant high-frequency activity and HFEOs, as determined by spectral analysis (Table 1). Thus, unlike seizure onset activity commonly reported for mesial temporal lobe onset seizures (Schiller *et al.*, 1998), this finding in an unselected group of patients (non-lesional and lesional MRI) supports the notion that high-frequency activity is common at the onset of focal neocortical seizures.

In all six (100%) patients with neocortical seizures, the seizure onset zone shows a significant increase in interictal high-frequency activity, both at baseline and during the pre-seizure period. This increase in high-frequency energy, unique to the seizure onset zone, comes from brief duration HFEOs, which have the same spectral characteristics as the seizure onset oscillations, as well as an increase in overall broadband high-frequency activity (Fig. 4). Even in the absence of distinct HFEOs during the interictal record, there is more broadband high-frequency energy within the seizure onset zone compared with control/normal/non-epileptogenic regions. Since the macroscopic field potentials recorded from subdural electrodes (Fig. 3) represent summated synaptic potentials of many neurons (Traub, 1999; Traub *et al.*, 2001), the increased broadband energy in the seizure onset zone probably represents an overall increase in asynchronous high-frequency neuronal activity, and the intermittent HFEOs are likely to be a network manifestation of increased neuronal synchrony. This is consistent with single neuron multiunit recordings obtained from human entorhinal cortex, which show an increase in single neuron bursting and neuronal synchrony in epileptic foci (Bragin *et al.*, 2002).

Importantly, we did not find HFEOs outside the seizure onset zone, supporting that these oscillations are pathological epileptic oscillations unique to epileptic brain. The frequencies of interictal HFEOs (Table 1) are well above the Berger EEG frequency bands (0.1–30 Hz), and are of such brief duration and low amplitude that utilizing common clinical EEG viewing parameters (e.g. 10 s page and 0.5 mV/mm), these oscillations are obscured by lower frequency range activity. In fact, even when occurring as a sustained discharge at the seizure onset, the HFEO is not always clearly apparent (Fig. 2) (Allen *et al.*, 1992; Fisher *et al.*, 1992; Alarcon *et al.*, 1995; Traub *et al.*, 2001).

The increase in high-frequency energy within the seizure onset zone during sleep compared with wakefulness was found in all six patients, but only reached statistical significance in three patients. This is a robust finding, given that the probability of finding increased high-frequency energy in the seizure onset zone in all six subjects by chance would be of the order of ~1.5%. The increase of HFEOs and broadband high-frequency activity within the seizure onset zone during slow-wave sleep demonstrates that neocortical networks in the seizure onset zone are strongly modulated by the behavioural state. The increase of high-frequency activity within a neocortical seizure onset zone is consistent with single and multiunit recordings in epileptic entorrhinal cortex which show an increase in high-frequency neuronal bursting and increased synchrony during slow-wave sleep (Steriade *et al.*, 1995; Staba *et al.*, 2002*a*, *b*). The significant increase in high-frequency activity in the seizure onset zone during slow-wave sleep may explain the well-known clinical observation that neocortical seizures frequently occur during sleep (Herman *et al.*, 2001).

Evidence for a fundamental role for high-frequency activity in the initiation of neocortical seizures (ictogenesis) is suggested by the localized spatial distribution of HFEOs and the frequent increase in interictal high-frequency activity within the seizure onset zone prior to the onset of seizures. The increase in high-frequency activity prior to seizures was found in 21 (62%) of the 34 neocortical seizures analysed. The seizures preceded by a statistically significant increase in high-frequency activity are shown in the Table 1. It is possible that limited spatial sampling due to clinical necessity (clinical morbidity rises with increasing number of implanted electrodes) may have precluded picking up this activity during the other seizures, in which the seizure onset zone may not have been completely covered with intracranial electrodes. A limitation of the present method is the relative lack of temporal resolution for identifying significant changes in the high-frequency energy in the pre-seizure period. In order to obtain statistical significance and refute the null hypothesis, that there is no difference in mean high-frequency energy between the pre-seizure and interictal periods, interictal and pre-seizure periods of the order of 20 min are required. This precludes the possibility of actual 'seizure prediction', but may provide a useful measure of increased probability of seizure occurrence.

Whether increased broadband high-frequency activity in the seizure onset zone is the manifestation of a dynamic state where the threshold for seizure onset is lowered (possibly in the same way that the behavioural state modulates high-frequency activity) is an important question that cannot be answered directly by these results. Animal (Bragin *et al.*, 1995; Maloney *et al.*, 1997) data have demonstrated increased gamma frequency (30–60 Hz) activity during wakefulness and paradoxical sleep compared with slow-wave sleep. Similarly, magnetoencephalography (MEG) (Llinas and Ribary, 1993) and EEG (Gross and Gotman, 1999) studies in humans have demonstrated that gamma

activity is highest during wakefulness and rapid eye movement sleep compared with slow-wave sleep. Gross and Gotman (1999) demonstrated that gamma activity during mental activation was increased further when performing cognitive tasks compared with quiet wakefulness. Many studies have examined gamma frequency activity at the intracellular level (Llinas *et al.*, 1991; Steriade and Amzica, 1996; Steriade *et al.*, 1996, 1998; Penttonen *et al.*, 1998) and demonstrated gamma frequency activity in all behavioural states, but enhanced during brain activation (rapid eye movement sleep and wakefulness).

Fast oscillations (>100 Hz), called ripple oscillations, have been described in rat hippocampus and peririhinal cortex, where they are associated with the bursts of sharp potentials during anaesthesia, behavioural immobility and sleep (Buzsaki *et al.*, 1992; Chrobak and Buzsaki, 1996). Ripple oscillations were also described in cat cortex during anaesthesia and natural states of vigilance (Grenier *et al.*, 2001) and recently have been implicated in an experimental model of seizures in cats (Grenier *et al.*, 2003). During slow-wave sleep and anaesthesia, ripple oscillations are selectively correlated to the depolarizing phase of the slow field oscillation (~1.0 Hz). During natural states of vigilance, ripple oscillations are more prominent during the depolarizing phase of slow wave sleep than during wakefulness and rapid eye movement sleep. Ripple oscillations have been described recently in human hippocampus and entorhinal cortex (Bragin *et al.*, 1999*a*,*b*; Bragin *et al.*, 2002), and demonstrated to be modulated similarly by the behavioural state, i.e. more prominent during slow-wave sleep compared with wakefulness or rapid eye movement sleep. Based on the intracellular recordings during different behavioural states, Steriade (Steriade and Amzica, 1996; Steriade *et al.*, 1996, 1998) has suggested that high-frequency EEG activity (>30 Hz) reflects the degree of depolarization of the neuronal membrane.

We might speculate that the increased high frequency activity seen in the minutes prior to onset of some neocortical seizures is the result of an aberration of the same physiological mechanisms underlying memory consolidation, possibly synaptic modification, and progressive reinforcement of local epileptogenic circuits. The HFEOs are similar to ripple oscillations, in that they are increased during the relatively depolarized state of slow-wave sleep.

It is important to note that while slow-wave sleep does result in an increase of broadband high-frequency activity and HFEOs in the seizure onset zone, a simple change of behavioural state, i.e. from wakefulness to sleep, does not explain our findings prior to seizure onset. The majority of seizures analysed occurred without clear evidence of any behavioural state change by video and clinical EEG (1–20 Hz) criteria. However, more subtle state changes involving the same thalamocortical networks that modulate the behavioural state (Steriade *et al.*, 1993; Buzsaki *et al.*, 1992; Steriade and Contreras, 1995; Chrobak and Buzsaki, 1996; Staba *et al.*, 2002*a*, *b*), gamma oscillations and ripple oscillations are interesting potential contributors to these findings that warrant further study.

Previous studies have examined the spatiotemporal distribution of interictal epileptiform spikes and sharp waves prior to seizure onset and found that there were no changes in this activity prior to seizures (Gotman and Marciani, 1985; Katz *et al.*, 1991). In addition, it is well established that spike and sharp wave discharges are broadly distributed, often occurring well outside the focal seizure onset zone and even in the contralateral hemisphere (Engel, 1987; Blume *et al.*, 2001). These findings are in sharp contrast to our results, and suggest that the neuronal network mechanisms responsible for interictal HFEOs, and spikes and sharp waves are likely to be different.

We have been careful to eliminate high-frequency artefact from our records, and it is unlikely that any of the results reported here are due to measurement artefacts. All calculations were performed using a bipolar montage, which should help eliminate referential and other global artefacts. We find that the HFEOs are unique to the seizure onset zone in all patients, and that the high-frequency activity is increased during sleep, both of which argue against muscle or environmental artefacts. During the immediate post-ictal period, when the background EEG activity is severely suppressed, we did not observe HFEO or high-frequency activity in the seizure onset zone, which also supports a physiological basis for HFEOs.

Conclusions

HFEOs demonstrate a high degree of spatial localization to the seizure onset zone, contrary to epileptic spikes and sharp waves, indicating that HFEOs could prove clinically useful for more accurate localization of the seizure onset zone. Unfortunately, only in two patients was there adequate information available about the relationship of electrode sites and the brain region resected at surgery.

Broadband high-frequency activity and HFEOs in the seizure onset zone are strongly modulated by behavioural state, and are most prominent in slow-wave sleep, which provides insight into how sleep states differentially modulate abnormal epileptic neuronal networks in human neocortical epilepsy. The most important finding is that focal neocortical seizures frequently occur during periods of increased high-frequency activity in the seizure onset zone, supporting the idea that broadband high-frequency activity and HFEOs are involved in neocortical ictogenesis.

These findings indicate that increased broadband high-frequency activity and HFEOs are signatures of epileptic brain, and that a more complete understanding of their role in ictogenesis may lead to a new understanding of mechanisms underlying epileptogenesis in the neocortex and novel treatments for these frequently medicine-resistant patients (Litt *et al.*, 2001; Litt and Lehnertz, 2002).

We have initiated a prospective clinical study to investigate further the clinical utility of using HFEOs to localize the

seizure onset zone for epilepsy surgery, and to evaluate the possibility of real-time identification of periods of increased seizure probability in neocortical epilepsy.

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