

Time–frequency analysis of single pulse electrical stimulation to assist delineation of epileptogenic cortex

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Epilepsy surgery depends on reliable pre-surgical markers of epileptogenic tissue. The current gold standard is the seizure onset zone in ictal, i.e. chronic, electrocorticography recordings. Single pulse electrical stimulation can evoke epileptic, spike-like responses in areas of seizure onset also recorded by electrocorticography. Recently, spontaneous pathological high-frequency oscillations (80–520 Hz) have been observed in the electrocorticogram that are related to epileptic spikes, but seem more specific for epileptogenic cortex. We wanted to see whether a quantitative electroencephalography analysis using time–frequency information including the higher frequency range could be applied to evoked responses by single pulse electrical stimulation, to enhance its specificity and clinical use. Electrocorticography data were recorded at a 2048-Hz sampling rate from 13 patients. Single pulse electrical stimulation (10 stimuli, 1 ms, 8 mA, 0.2 Hz) was performed stimulating pairs of adjacent electrodes. A time–frequency analysis based on Morlet wavelet transformation was performed in a [–1 s : 1 s] time interval around the stimulus and a frequency range of 10–520 Hz. Significant ($P = 0.05$) changes in power spectra averaged for 10 epochs were computed, resulting in event-related spectral perturbation images. In these images, time–frequency analysis of single pulse-evoked responses, in the range of 10–80 Hz for spikes, 80–250 Hz for ripples and 250–520 Hz for fast ripples, were scored by two observers independently. Sensitivity, specificity and predictive value of time–frequency single pulse-evoked responses in the three frequency ranges were compared with seizure onset zone and post-surgical outcome. In all patients, evoked responses included spikes, ripples and fast ripples. For the seizure onset zone, the median sensitivity of time–frequency single pulse-evoked responses decreased from 100% for spikes to 67% for fast ripples and the median specificity increased from 17% for spikes to 79% for fast ripples. A median positive predictive value for the evoked responses in the seizure onset zone of 17% was found for spikes, 26% for ripples and 37% for fast ripples. Five out of seven patients with <50% of fast ripples removed by resection had a poor outcome. A wavelet transform-based time–frequency analysis of single pulse electrical stimulation reveals evoked responses in the frequency range of spikes, ripples and fast ripples. We demonstrate that time–frequency analysis of single pulse electrical stimulation can assist in delineation of the epileptogenic cortex using time–frequency single pulse-evoked fast ripples as a potential new marker.

Keywords: epilepsy; electrocorticography; single pulse electrical stimulation; high-frequency oscillations; time–frequency analysis

Abbreviations: ERSP = event-related spectral perturbation; PPV = positive prediction value; SPES = single pulse electrical stimulation

Introduction

Delineation of the epileptic cortex remains the greatest challenge in epilepsy surgery. Despite a tremendous increase in the number of antiepileptic drugs that have become available over the past few decades, ~20–30% of the patients with epilepsy are not satisfactorily controlled with antiepileptic drugs. Resective surgery is a potentially curative option for 12.5% of these patients with refractory focal epilepsy (Valentín *et al.*, 2002; Lesser *et al.*, 2010). Unfortunately in these patients, the area responsible for seizure generation, represented by a hypothetical epileptogenic zone, is difficult to define precisely. Although several preoperative techniques, for example interictal and ictal EEG, PET, MRI, single-photon emission computed tomography and magnetoencephalography, are available, these are still time consuming and only partly accurate (Morris *et al.*, 2008; Lesser *et al.*, 2010). New reliable pre-surgical localizers of epileptogenic tissue are therefore still welcome to guide the resection, improve outcome and avoid ineffective surgery.

In difficult cases, intracranial EEG evaluation is performed ultimately to identify the onset of spontaneous seizures (seizure onset zone) and localize the cortical regions responsible for their generation while preserving the eloquent brain areas. The success of these chronic subdural electrocorticography registrations depends on the occurrence of the patient's typical seizures (Carreño and Lüders, 2008; Lesser *et al.*, 2010). Waiting for spontaneous occurrence of seizures makes this a 'long-term' invasive procedure. Other biomarkers for the epileptogenic zone have been suggested, such as interictal spikes and the more recently discovered spontaneous high-frequency oscillations. Interictal spikes are related to the epileptogenic zone, but their specificity is not very high (Lesser *et al.*, 2010). Recently, it has been found that spontaneous high-frequency oscillations (80–500 Hz) specify the epileptogenic zone better than spikes. Generally, these high-frequency oscillations can be divided into ripples (80–250 Hz) and fast ripples (250–500 Hz) (Bragin *et al.*, 1999, 2002; Jacobs *et al.*, 2008). Total removal of high-frequency oscillation generating tissue, in particular that which generates fast ripples, correlates with a good surgical outcome (Jacobs *et al.*, 2010). Still, the occurrence of high-frequency oscillations is unpredictable and their visual detection is extremely time consuming (Jacobs *et al.*, 2008).

To reduce invasive monitoring time, with its risks of complications and patient discomfort, one wishes to be independent of random spontaneous events such as spikes, high-frequency oscillations and seizures. Valentín *et al.* (2002) demonstrated that electrocortical stimulation with brief single pulses (single pulse electrical stimulation; SPES) can evoke epileptiform transients resembling EEG spikes (Valentín *et al.*, 2002). The location of these evoked epileptic responses is related to the seizure onset zone and removal of this area is associated with better surgical outcome for temporal and frontal lobe epilepsy (Valentín *et al.*, 2002, 2005a, b; Flanagan *et al.*, 2009). A recent finding in an animal model is that single pulse stimulation can also evoke epileptic high-frequency oscillations (Rolston *et al.*, 2010). SPES evoked high-frequency oscillations have not yet been studied for their clinical significance.

The main advantages of the SPES technique are that it can be scheduled and does not require long-term monitoring. Moreover, though invasive, it is a passive and non-painful test for the patient. However, the clinical use of SPES is compromised by the subjective visual analysis in the time domain and the use of statistics. The latency, waveform definitions and decision of what constitutes an epileptic response, are fairly indistinct. Valentín *et al.* (2002) proposed a cumulative evaluation of the responses after 10 stimuli in a statistical way, i.e. in relation to the occurrence of pre-stimulus spontaneous spikes (Valentín *et al.*, 2002). Review of the expected evoked high-frequency oscillation responses in the time domain by the method described by Jacobs *et al.* (2008) would complicate visual evaluation of SPES responses even further.

These drawbacks emphasize the need for an alternative analysis method. We introduce a quantitative EEG analysis method that allows time as well as frequency evaluation of SPES responses. This time–frequency analysis is based on wavelet transformation, and also automatically takes statistics into account. In a retrospective study, electrodes showing time–frequency SPES evoked responses in different frequency ranges are compared with the seizure onset zone and surgical outcome in a number of patients undergoing long-term invasive monitoring. We wanted to see if our time–frequency SPES analysis would provide a new tool for the delineation of the epileptogenic cortex in these patients.

Materials and methods

Patients

Chronic electrocorticography data were recorded from 13 patients (seven males, six females, mean age 21 years, range 8–42 years) with intractable focal epilepsy who underwent subdural electrode grid monitoring for epilepsy surgery evaluation. Patients were admitted to the Intensive Epilepsy Monitoring Unit of the University Medical Centre of Utrecht, The Netherlands, between 2008 and 2010. The patients were monitored for 5–7 days. The SPES protocol was routinely performed as from 2004, and recorded at the sampling rate of 2048 Hz on a regular base as from 2008. For all 13 patients, this monitoring resulted in resection of a presumed epileptic focus. The conventional visually analysed SPES results, in line with recommendations of Valentín *et al.* (2002), were included in clinical decision making. There were three patients with temporal, three with frontal, one with parietal, three with frontocentral, one with centroparietal, one with central and one with occipital lobe epilepsy. Most patients were on multiple antiepileptic drugs that were tapered during the registration. Patient information is given in Table 1. The procedure was approved by the hospital's Medical Ethics Committee.

Electrocorticography

Chronic electrocorticography was performed with subdural electrodes (Ad-Tech) that were placed through craniotomy. These electrodes were divided over four blocks of each 32 electrode connections. Subdural grids and strips consisted of platinum circular electrodes with 4.2 mm² contact surface, embedded in silicone, with an interelectrode distance of 1 cm. In one patient, two depth electrode strips were also implanted, each with eight cylindrical contacts with 7.9 mm² contact surface and 5 mm interelectrode distance (Ad-Tech). For

Table 1 Individual patient characteristics

Patient	Age/sex	Localization	Side	Pathology	Electrodes	Recording days	Engel Class
1	31/F	Temporal	R	Malformation of cortical development	88	7	I
2	23/F	Temporal	L	Glioneural heterotopia	96	8	–
3	29/M	Parietal	L	Lesion tumour resection at age of 4	72	4	–
4	9/M	Frontal	L	Focal cortical dysplasia	96 (16 depth)	5	IV
5	11/M	Central	R	Focal cortical dysplasia	72	9	–
6	26/F	Occipital	L	Gliosis and old infarctions	96	8	–
7	42/M	Temporal	L	Mesiotemporal sclerosis	96	7	I
8	25/F	Temporal occipital	L	Tumour	80	7	I
9	13/F	Frontocentral	L and R	Tuberous sclerosis (multiple tubers)	96	5	IV
10	13/M	Frontal	L	Focal cortical dysplasia	78	6	IV
11	8/M	Frontocentral	L	Tumour	96	5	I
12	22/F	Centroparietal	R	–	104	6	III
13	23/M	Frontocentral	R	Malformation of cortical development	128	4	III

F = female; L = left; M = male; R = right.

each patient, various subdural electrode grids and strips were placed covering the brain areas suspected for epileptic activity and bordering functional areas in the affected hemisphere. In the 13 patients, the number of implanted electrodes ranged from minimal 72 to maximal 128 electrodes, with on average 93 electrodes per patient. One patient had a grid placed on the left hemisphere and electrode strips placed bilaterally.

Intracranial electrodes and clinical data

For all patients, the electrodes covering the seizure onset zone and the resected area of the eventual surgery were marked by the involved clinical neurophysiologists (C.H.F., F.S.S.L.). They were aware of the conventional visual analysed SPES results as part of our clinical practice, but unaware of the time–frequency SPES study results that became available only after surgery. The exact electrode positions were determined by digital photographs made during implantation, and with a post-implantation CT scan showing electrode artefacts, which was matched to an MRI surface rendering acquired preoperatively (Noordmans *et al.*, 2002). The seizure onset zone was defined as the set of electrode contacts that showed the earliest ictal activity in the electrocortigram. Early ictal activity was defined as the first electrocortigram pattern consisting of rhythmic spikes, rhythmic sharp waves, spike and slow waves complexes, increasing gamma activity, regular or sharpened delta or theta activities, or low-amplitude activity in the beta range (Alarcon, 1996; Jung *et al.*, 1999; Valentin *et al.*, 2002) preceding or coinciding with a habitual clinical seizure.

For patients with a follow-up >12 months or immediate continuation of the seizures, the outcome of surgery was determined (Engel classification) (Engel *et al.*, 1993). An Engel class I was considered a good post-surgical outcome and Engel classes II–IV were considered as poor post-surgical outcome.

Single pulse data acquisition

Data were recorded at a sampling rate of 2048 Hz, using a hardware anti-aliasing filter of 538 Hz. Recording at this high sample rate allowed only 64 channels to be recorded. The selection of the 64 recorded electrodes was based on the monitoring results of the previous days, including the clinical seizure onset zone. A manually

controlled cortical stimulator was used (IRES 600, Micromed). Monophasic SPES stimuli, 10 pulses with a duration of 1 ms, an intensity of 4–8 mA at a frequency of 0.2 Hz were given on pairs of adjacent electrodes, systematically proceeding over the selected grids. While executing the SPES protocol the guidelines described by Valentin *et al.* (2002, 2005b) were followed: stimulation was started at 8 mA and only in stimulation pairs where twitches or pain occurred, the intensity was gradually reduced as low as 4 mA.

Preprocessing

The preprocessing of the data files was performed with dedicated software implemented in Matlab (Mathworks). The data were not initially recorded keeping the proposed analysis method in mind. Therefore, a preprocessing step detecting exact stimulus timing was required. Other steps included stimulus artefact correction, re-referencing to average reference and epoching of the data. Linear interpolation of the data in all electrodes in the [–0.9 ms : 8 ms] interval surrounding the stimulus was performed to correct for the saturation effect induced by the amplifier. An epoch interval of [–1 s : 1 s] covering pre-stimulus baseline and post-stimulus response interval was chosen. This resulted in 10 epochs of 2 s for each pair of stimulated electrodes. For subsequent computations, a subinterval of [–1 s : –0.2 s] was defined as computational baseline to avoid interference of the residual stimulus artefact into the baseline period. No additional filtering was performed on the data.

Time–frequency analysis

A wavelet-based time–frequency transformation was applied to the epoched data. For this wavelet analysis, the implementation in the Matlab toolkit EEGLab developed by Delorme and Makeig (2004) was used. This implementation uses a Morlet wavelet with best performing default settings consisting of a sliding latency window size of 685 samples (334.5 ms), a window overlap of 50% and two oscillation parameters, 3 and 0.5, respectively. The frequency range of interest was set to 10–520 Hz, with a frequency resolution of 1 Hz. The lower frequency limit is determined by the chosen wavelet window settings. Taking the window size into account, the remaining time interval of 1665.5 ms was divided into 200 samples, resulting in a time resolution

of 8.3 ms. The averaged logarithmic power spectrum (dB) of 10 epochs was computed for the given frequency range and epoch interval.

Significance levelled event-related spectral perturbation

The calculated mean baseline power was subtracted from each spectral estimate to address the changes in power evoked by stimulation instead of absolute power. This is known as the event-related spectral perturbation (ERSP; Delorme and Makeig, 2004).

A 'bootstrapped' significance was calculated to determine the statistical significance of the power changes after stimulation for each ERSP. Bootstrapping addresses the significance of deviations from the baseline power by randomly re-sampling ($n = 200$) the spectral estimates of the selected pre-stimulus epoch data of each trail and then averaging these, thus constructing a surrogate baseline data distribution. The ERSP was tested with respect to an assumed surrogate data distribution with a chosen significance level ($P = 0.05$). Non-significant ERSP values were discarded by setting them to zero (Delorme and Makeig, 2004).

Event-related spectral perturbation image

The resulting significant ERSP constituted a matrix containing 511 rows representing the total frequency range and 200 time points representing the total epoch. This matrix was calculated for all 64 recorded channels for each stimulated electrode pair, and visualized as an ERSP image using a uniform [−15 dB : 15 dB] colour scale with saturation of values above the limit of ± 15 dB (accommodating the stimulus artefact). Note that this colour scaling shows changes in power relative to the baseline for each frequency. In Fig. 1, a schematic representation, by means of a flow chart, of the different steps in this time–frequency analysis method, from now on called time–frequency SPES, is given.

Event-related spectral perturbation visual analysis

The time–frequency SPES evoked responses present in the ERSP images (64 electrodes \times stimulated electrode pairs) were visually classified. A time region of interest was defined as the response period [0.1 s : 1 s] after stimulation, based on the latency definitions of Valentin *et al.* (2002, 2005a, b) for 'delayed pathologic responses'. For this response period, responses were classified into three frequency regions of interest: 10–80 Hz, representing epileptiform low frequency transients (the delayed pathologic responses) hereafter termed the 'spike' band, 80–250 Hz, referred to as the 'ripple' band and 250–520 Hz, referred to as the 'fast ripple' band. In a joint meeting, the observers were shown several examples of ERSP images and corresponding delayed responses and high-frequency oscillations as visually marked in the raw time domain SPES data for one patient by an experienced observer (M.Z.), following the methodology of Jacobs *et al.* (2008). The definition of a response was based on these example images, and defined as a clear increase (orange–red) in power. In case a clear increase in power was present in the baseline period, the response had to be of higher intensity in order to be marked as response. Observers were instructed to discard artefacts, such as the

spectral scattering > 80 Hz due to muscle artefacts (Otsubo *et al.*, 2008; Zijlmans *et al.*, 2011).

Then visual classification of time–frequency SPES evoked responses in all ERSP images was performed by two observers (C.H.F., F.S.S.L., G.J.M.H. or M.A.vtK.) independently. Inter-observer agreement was calculated using Cohen's kappa (κ) using SPSS 18 (PASW Statistics 18, Rel. 18.0.0, 2009, SPSS Inc.). The κ -scores were calculated separately for the number of responses classified as spike, ripple and fast ripple. A $\kappa > 0.4$ was interpreted as sufficient agreement (Zijlmans *et al.*, 2002). For the final counts of spikes, ripples and fast ripples per patient, only responses for which there was agreement between the two independent observers were accepted. The total amount of responses was calculated for spikes, ripples and fast ripples and for each recorded electrode. Data sets with $\kappa < 0.4$ for all three frequency regions were excluded from further analysis.

Statistical analysis

Statistical analysis was performed for spikes, ripples and fast ripples separately and for each individual patient. The correspondence between electrodes showing a time–frequency SPES evoked response was related to the clinically defined seizure onset zone electrodes. The populations' median sensitivity, specificity, positive (PPV) and negative prediction value (NPV) of time–frequency SPES evoked responses for the seizure onset zone were calculated. These prediction values are calculated with the electrodes defined as positive or negative with regard to the presence of evoked responses and involvement in seizure onset, resulting in PPV_{el} and NPV_{el} .

PPV_{el} is defined as

$$\frac{\text{number of positive electrodes within SOZ}}{\text{number of positive electrodes}}$$

NPV_{el} is defined as

$$\frac{\text{number of negative electrodes within SOZ}}{\text{number of negative electrodes}}$$

where SOZ is the seizure onset zone.

The PPV was also calculated for the time–frequency SPES responses elicited by all stimulated electrode pair sites (PPV_{resp}), thus weighing the number of evoked responses on a positive electrode.

PPV_{resp} is defined as

$$\frac{\text{number of evoked responses on positive electrodes within SOZ}}{\text{total number of evoked responses on all positive electrodes}}$$

Results

In Fig. 2, two examples are given of the correspondence for the same single epoch of an evoked response as analysed by time–frequency SPES and the conventional visually marked spikes, ripples and fast ripples and an expanded view of these oscillations. These were used in the joint meeting preceding the visual classification of time–frequency SPES evoked responses of all data by the independent observers.

In 13 patients, a median of 46 (range 16–56) electrode pairs were stimulated and the ERSP images of 64 electrodes for each stimulated electrode pair were evaluated, yielding a median of 2944 (range 1024–3584) ERSP images. In Fig. 3, three examples are given of significant averaged ERSP images classified as no response, a time–frequency SPES evoked spike response only, and a

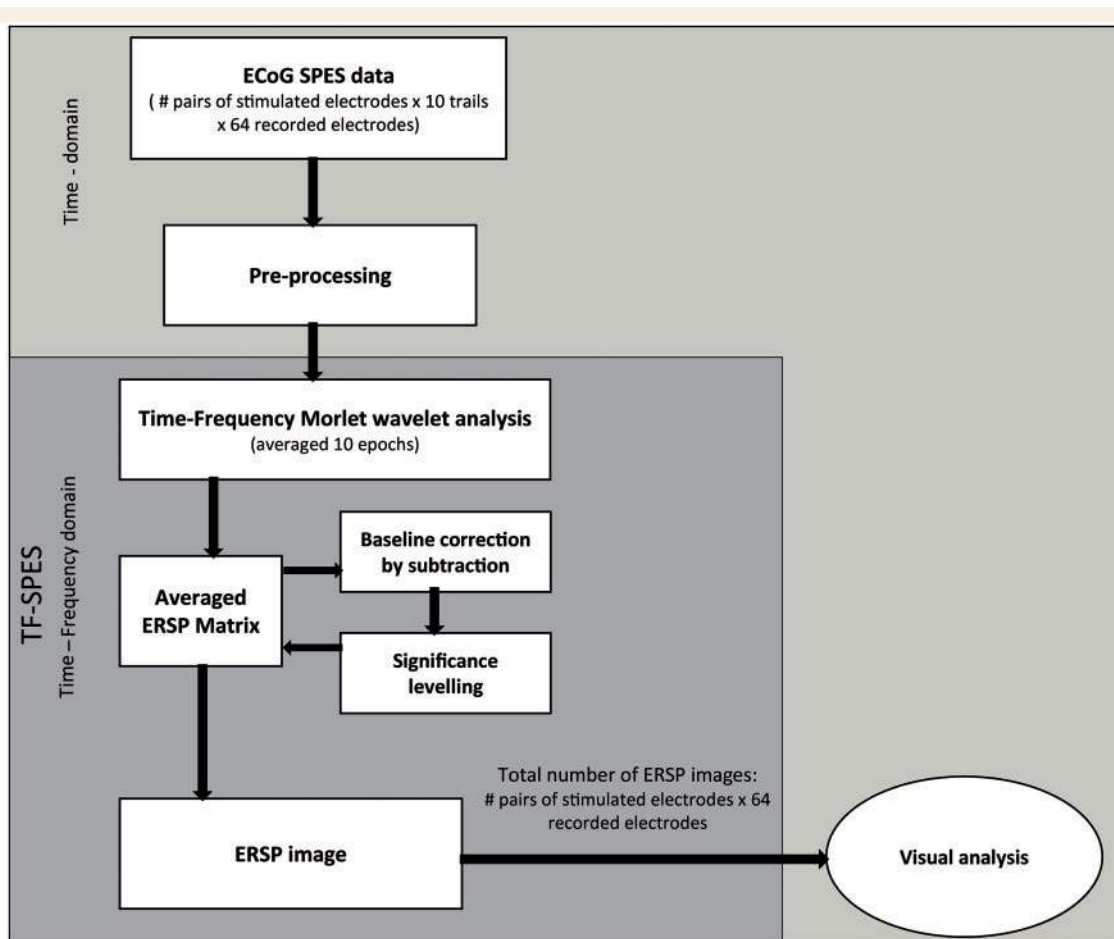


Figure 1 Flow chart of different steps in the time–frequency analysis. ECoG = electrocorticography.

combined evoked spike, ripple and fast ripple response. Note that these examples are chosen from results of different patients.

Visual classification of time–frequency SPES evoked responses was performed for all ERSF images of the 13 included patients. The inter-observer agreement for one patient (Patient 3) resulted in a $\kappa < 0.4$ for spikes, ripples and fast ripples, and these data were rejected from further analysis. Thus, inter-observer consensus scores for spikes, ripples and fast ripples remained for 12 patients and were further analysed. Within this group of 12 analysed patients, the inter-observer agreement for five patients demonstrated a $\kappa < 0.4$ for solely one frequency range, but were still analysed. For one patient, this concerned the spike band, and for four patients this concerned the fast ripple band.

In total, 6553 responses were classified for 12 patients. For all 12 patients, each of three defined responses spikes, ripples and fast ripples were found. The median number per patient of spikes was 320 (range 75–790), of ripples 125 (range 26–465) and of fast ripples 29 (range 2–112).

Relation of spikes, ripples and fast ripples with the seizure onset zone

For the 12 analysed patients, the median number of seizure onset zone electrodes of the ictal electrocorticography was five (range

0–30). No clear seizure onset zone could be identified in one patient. In the remaining 11 patients, the median sensitivity of time–frequency SPES for seizure onset zone decreased and its median specificity increased with higher frequency range. There is a high sensitivity (100%) and low specificity (17%) for spikes versus a lower sensitivity (67%), but higher specificity (79%) for fast ripples. A median PPV_{el} value of 17% was found for spikes, 19% for ripples and 27% for fast ripples. The PPV_{resp} improves this PPV_{el} by 7% for ripples and by 10% for fast ripples, resulting in a PPV_{resp} of 37% for fast ripples. There was no difference for spikes. This effect is illustrated in Figs 4 and 5, where time–frequency SPES responsive electrodes are classified as highly responsive (defined as electrodes with $\geq 50\%$ of the maximal number of responses present on a single electrode) and moderately responsive ($< 50\%$) for spikes, ripples and fast ripples. The median NPV_{el} was 100, 100 and 96% for spike, ripples and fast ripples, respectively.

Relation of seizure onset zone, spikes, ripples and fast ripples with outcome

The post-surgical outcome after 1 year was available in nine patients; the other patients had been operated on too recently. Four patients (Patients 1, 7, 8 and 11) had good surgical outcome in

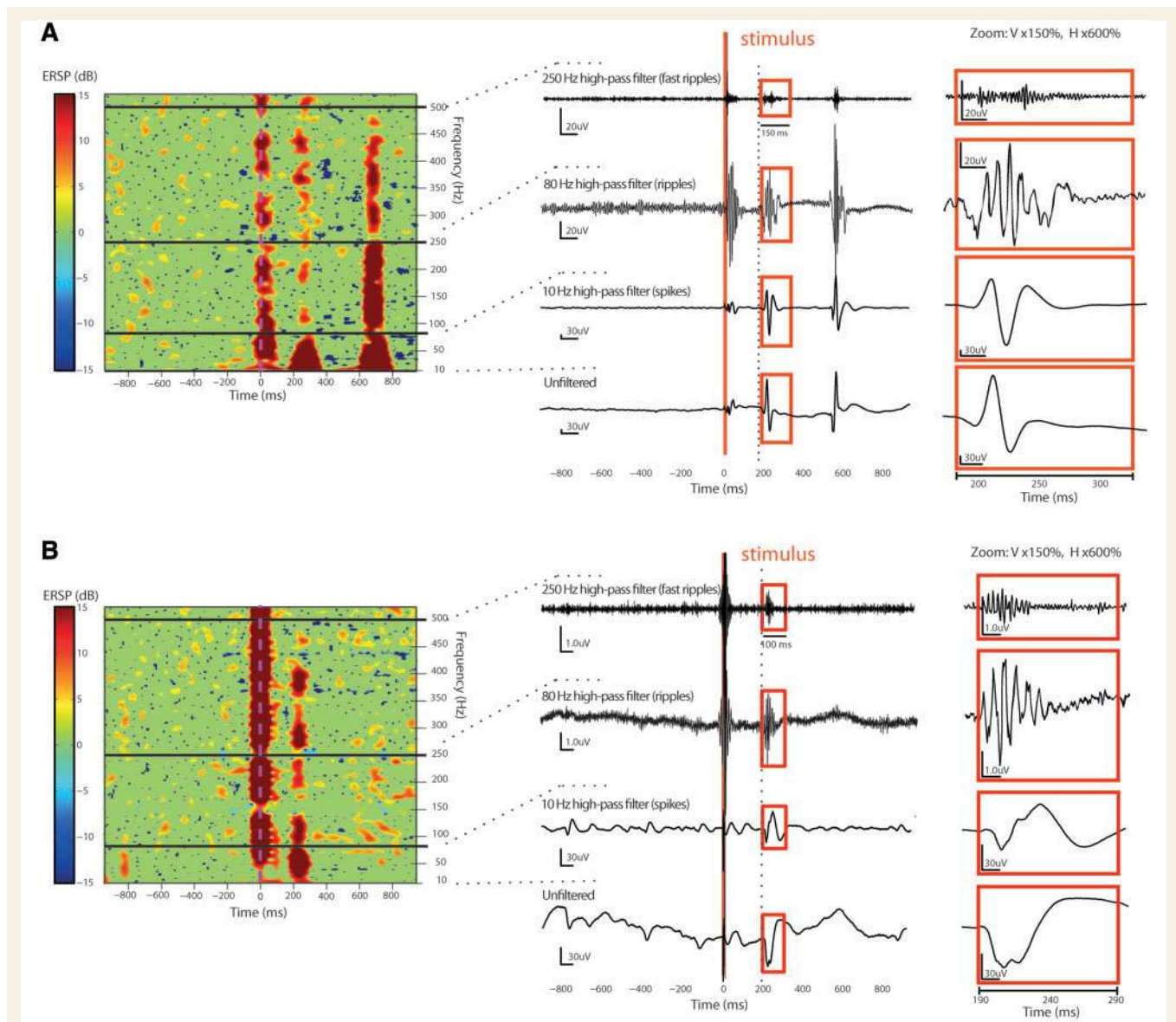
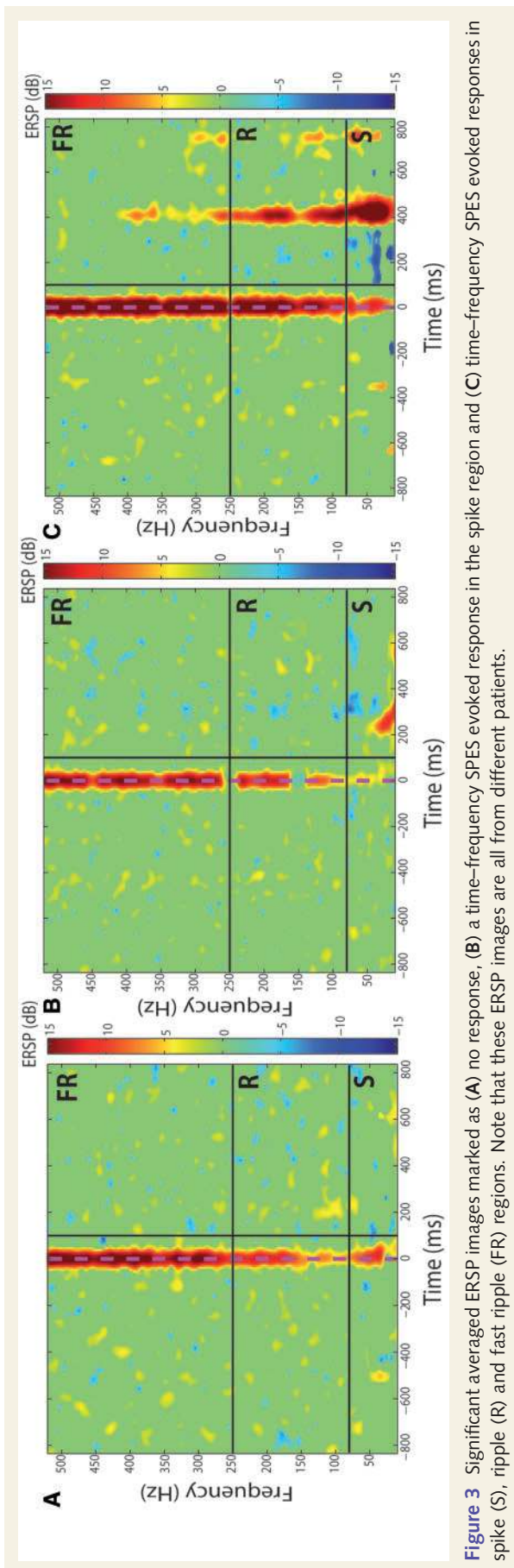


Figure 2 Two examples (**A** and **B**) of the correspondence of the same single epoch between (*left*) a significant ERSP image as result of time–frequency SPES analysis and (*centre*) time domain electrocorticography data, high pass filtered (Stellate Harmonic FIR filter, order 63) for spike band (>10 Hz), ripple band (>80 Hz) and fast ripple band (>250 Hz), and the unfiltered signal (*right*) a close up of the evoked oscillations in each frequency band. The example in **A** (Patient 1) contains, at ~200 ms, an evoked high amplitude spike (unfiltered 380 μ V) with superimposed ripple (72 μ V) and fast ripple (15 μ V) and a second larger spontaneous spike with accompanying large ripple and fast ripple. Note that the second spontaneous spike shows relatively more ripple component compared with the evoked spike, a phenomenon that is described as a potential side effect of filtering (Urresterazu *et al.*, 2007) in which false high-frequency oscillations show a significant ripple. The example in **B** (Patient 4) shows, at ~200 ms, an evoked low-amplitude spike (unfiltered 80 μ V) with superimposed ripple (4.3 μ V) and fast ripple (1.3 μ V). Both evoked spikes result in differentiated small blobs with significant fast ripple blobs in the ERSP, in the absence of large (elongated) blobs in the ripple band.

Engel class I, but were still treated with antiepileptic drugs. In five patients (Patients 4, 9, 10, 12 and 13), surgery resulted in an outcome of Engel class II–IV. In these nine patients, the resection area had been covered by a median number of nine electrodes (range 5–36). The seizure onset zone was totally resected in four patients (Patients 1, 7, 10 and 12), of which two (Patients 1 and 7) had a good surgical outcome of Engel class I. These two patients both suffered from temporal lobe epilepsy. Total removal of

the area showing time–frequency SPES evoked fast ripples, which best correlated with seizure onset zone, was retrospectively achieved in one patient (Patient 7) who showed good post-surgical outcome. The other patient with temporal lobe epilepsy (Patient 1) had 94% of the number of evoked fast ripples removed. Of the remaining seven patients, in whom <50% of evoked fast ripples were removed, five patients had a poor post-surgical outcome. Removal of area showing evoked spike



and ripples, which are less specific for the seizure onset zone, showed the same trends as fast ripples but with lower percentages.

Examples of time–frequency SPES evoked responses, seizure onset zone and resection area in four patients (Patients 1, 4, 10 and 13) are depicted in Figs 4 and 5. The results of the other nine patients are available in the Supplementary Material.

Discussion

We demonstrate a novel approach for the analysis of SPES based on wavelet transform time–frequency analysis. In this time–frequency SPES approach, the significance of evoked responses is incorporated in the results and data reduction is achieved by averaging of 10 trials into a single image. Time–frequency SPES shows that not only spikes, but also responses in the high-frequency oscillatory range can be evoked by SPES and can be readily recognized, whereas visual identification of spontaneous high-frequency oscillations in chronic electrocorticography is notoriously elaborate. The clinical value of the evoked responses, subdivided in a spike-, ripple- and fast ripple band, was shown. Our results show a trend that evoked fast ripples may be the most useful marker of the epileptogenic zone of the three. With a median specificity of 79% for the seizure onset zone and their incomplete removal being related to poor outcome, time–frequency SPES analysis assists in delineation of the epileptogenic cortex, with evoked fast ripples being the most promising new marker. If this promise is confirmed in larger studies, this will make interpretation of chronic electrocorticography independent from spontaneously occurring events, and thus shorten these invasive recordings.

Methodological aspects

Time–frequency analysis

In recent years, Morlet wavelet-based time–frequency decomposition has been proposed and used for the analysis of spectral properties of spontaneous interictal events like spikes and high-frequency oscillations in electrocorticography (Xiang *et al.*, 2009; Zemann *et al.*, 2009, 2010; Bénar *et al.*, 2010; Crépon *et al.*, 2010). In this literature, the discussion of how many false high-frequency oscillations are detected is often held. The signature of the Morlet wavelet for transients is different from oscillatory events: transients show large band elongated ‘blobs’ while oscillations show blobs with a more restricted frequency range (Bénar *et al.*, 2010). The Morlet Wavelet used in our time–frequency analysis resembles the Finite Impulse Response filter used in visual high-frequency oscillation detection following the methodology of Jacobs *et al.* (2008) and Bénar *et al.* (2010). In the ERSP images, we found evoked high-frequency oscillations (ripples, fast ripples) superimposed on spikes, but also spikes without concomitant ripples or fast ripples (Jacobs *et al.*, 2008; Crépon *et al.*, 2010), as is also the case in the time domain in spontaneous high-frequency oscillations. However, as is the case in spontaneous high-frequency oscillations, whether time–frequency SPES evoked power in the ripples and fast ripples band reflects specific properties of spikes or separate high-frequency oscillations activity

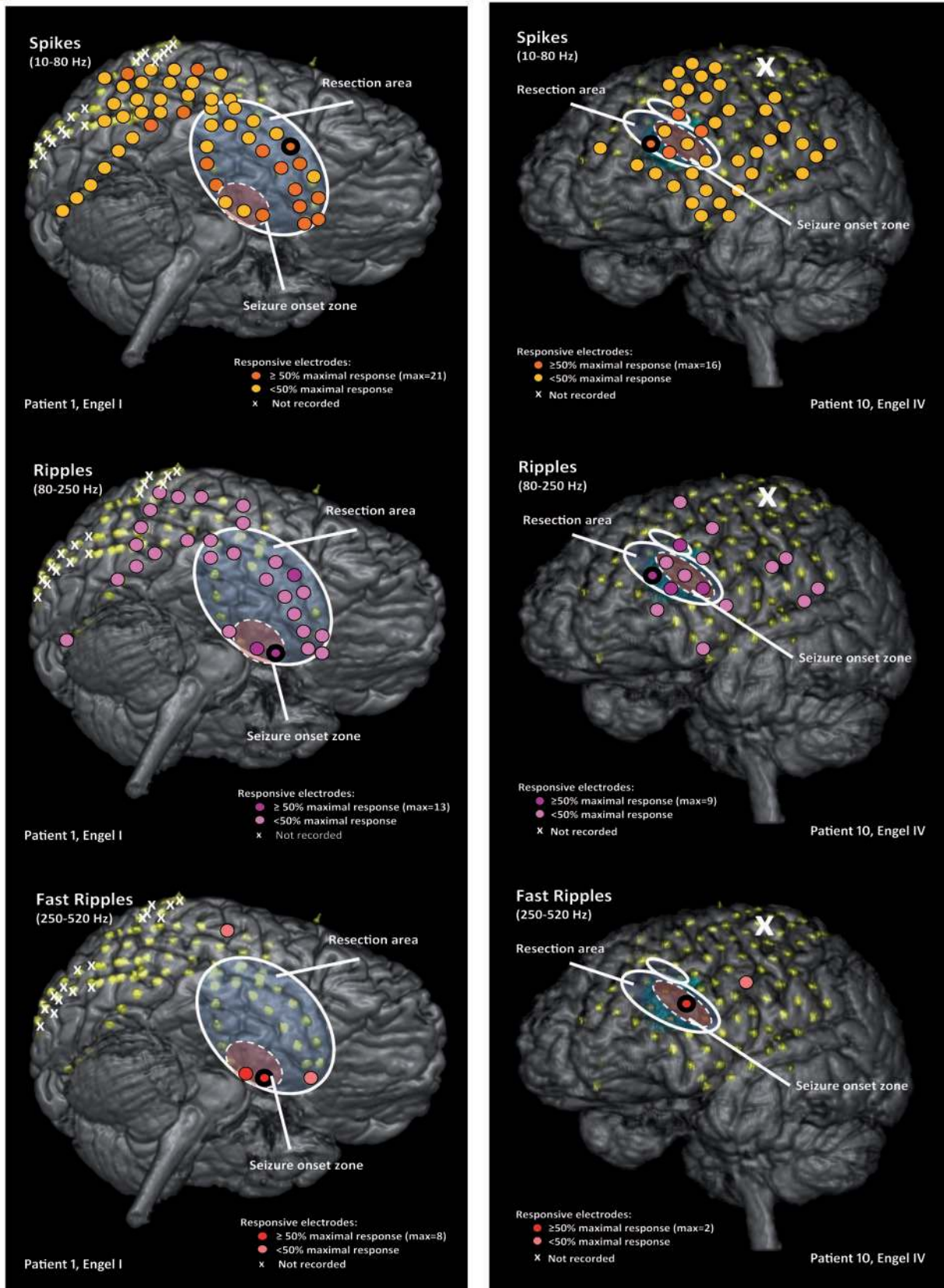


Figure 4 An example of total seizure onset zone removal by resection for Patient 1 (temporal lobe epilepsy) with good post-surgical outcome Engel class I (*left*), and Patient 10 with poor post-surgical outcome Engel class IV (*right*).

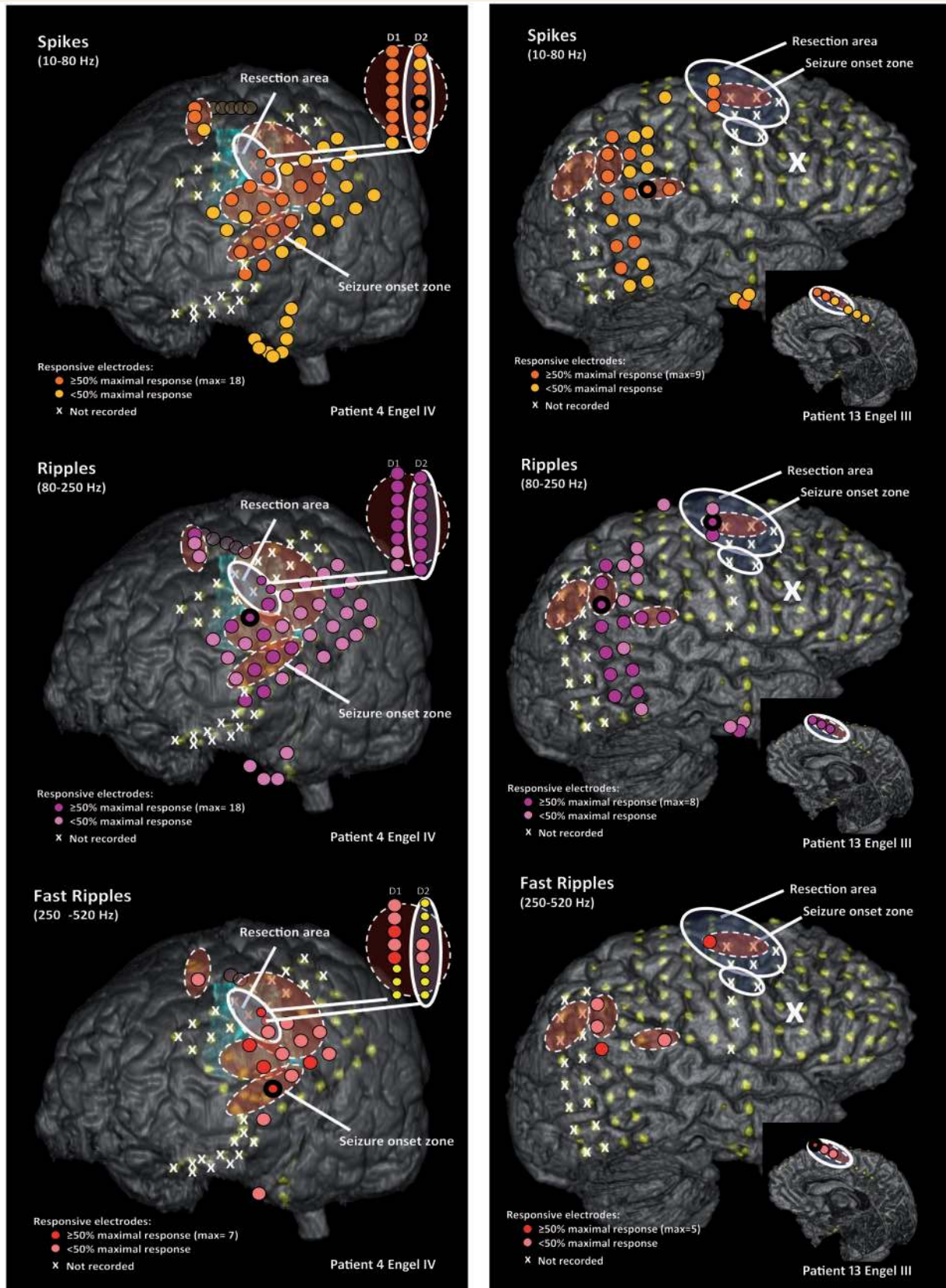


Figure 5 An example of incomplete seizure onset zone removal, incomplete fast ripple removal by resection and poor post-surgical outcome. Patient 4 (*left*), Engel class IV, D1 and D2 represent depth electrodes and Patient 13 (*right*), Engel class III.

is still under debate. Figure 2 shows two examples: in Fig. 2A a relatively steep (spontaneous) spike that can be characterized by its high power extending in the ripple and fast ripple band and an earlier evoked high-frequency oscillation that is a good candidate for one superimposed on a spike, because of the restricted frequency range of the blob and a smaller amplitude and steepness of the spike. The latter is also illustrated by the second example (Fig. 2B), with an even smaller amplitude and steepness of the initial evoked spike and relatively large fast ripple component. Urrestarazu *et al.* (2007) described that false high-frequency oscillation detection as a filter effect of sharp spikes reveals itself showing large ripple components superimposed on spikes. For practical purposes, regardless of the interpretation, the presence of significant power in the ripples and fast ripples band shows an increased specificity for the seizure onset zone in our data, which merits its clinical relevance.

Instead of doing a statistical test on 10 trials in order to discard spontaneous activity as being counted as evoked (as is done in the classical SPES time domain analysis), we chose to average responses and only consider classification of significant change in averaged spectral power. As a result, whether a classified event was based on 3 or 10 responses out of 10 stimuli is of no influence. In doing so, we avoid spurious detection of evoked responses at the expense of discarding possible sporadic events. So in terms of sensitivity and specificity, we sacrifice sensitivity and hope to gain specificity. In terms of epilepsy surgery, this would imply a conservative approach towards resecting tissue.

The added bootstrap significance threshold could not prevent some interference of spontaneous interictal activity in the data. Compared with this statistical correction method in Valentín and colleagues original paper (2002), our method seems less objective, but is more time efficient. Yet, the advantage of time–frequency SPES is that power spectra for multiple epochs are accumulated, and evoked responses with approximately the same latency show a larger power increase compared with spontaneous interictal events (Delorme and Makeig, 2004). Small jitter (~10 ms) between time-locked evoked responses in the time domain is therefore less of a problem in the frequency domain, also given that a moving window is used. The exact latency of responses was not further investigated or corrected for since it was no point of interest in this study. With the additional instruction for the observers that the intensity (redness in the ERSF image) of the evoked response after stimulation had to be larger than any event in the pre-stimulus baseline, we further limited the influence of spontaneous events in our results.

Event-related spectral perturbation analysis

In addition to the assigned significance by the bootstrapping method, visual classification is still involved in defining time–frequency SPES evoked responses. To avoid subjectivity, we took a conservative approach and let the relevance of scored spike, ripples and fast ripples be based on agreement between two independent observers. The κ -values of the inter-observer agreement resembled those for interictal discharges in magnetoencephalography and electrocorticography (Zijlmans *et al.*, 2002, 2007; Agirre-Arrizubieta *et al.*, 2009; Zelmann *et al.*, 2009). Some low κ -values were encountered and reflect poor signal-to-noise. Especially fast

ripples suffered from low κ in four patients. This is the case when few evoked responses are present, e.g. the discarded Patient 3 in whom the total number of evoked responses ($n = 43$) was far lower than the median number ($n = 320$) in our population. Probably, in the absence of clear events, a human observer subconsciously lowers the threshold for detection. We are in the process of constructing an algorithm to replace human observers and make time–frequency SPES fully automated.

Stimulation protocol

When SPES was first proposed by Valentín *et al.* (2002, 2005b) as a clinically useful tool, their results were described separately for patients with temporal lobe epilepsy and patients with frontal lobe epilepsy. They showed that the localization of the evoked events was most specific in temporal lobe epilepsy (Valentín *et al.*, 2002, 2005b). We performed SPES in different anatomical areas and showed that we could evoke late responses in the temporal and frontal lobe, as well as in central and parietal areas. Because we think that the inclusion of fast ripples is a most promising new finding, regional differences in the prediction of seizure onset zone and outcome may be different in time–frequency SPES from traditional SPES looking at spikes only. Our number of patients was insufficient to draw conclusions with regard to regional differences.

A further difference in the analysis of SPES responses compared with Valentín *et al.* (2002, 2005a, b) is that they established both association of pathology with the responsive electrodes and association with the site of stimulation that evoked a response (Valentín *et al.*, 2002, 2005a, b). At first sight in our data, we found no indication of a clear relation between the site of stimulation and the seizure onset zone. However, a thorough analysis requires a different approach based on the identification of epileptic networks, which is currently still not practical for surgery (David *et al.*, 2010).

Rolston *et al.* (2010) showed, in a more fundamental study, the existence of evoked high-frequency oscillations <100 ms after stimulation in an animal rat model (Rolston *et al.*, 2010). In this study, evoked high-frequency oscillations were investigated in the hippocampus and not in other cortical areas. Valentín *et al.* (2002) described, in a clinical study in a human population, responses in this same time interval as normal physiological responses, although the mechanisms of their generation are still unknown. In our clinical study, we wanted to process the SPES protocol and resulting data in line with the rationale of Valentín *et al.* (2002, 2005a, b) who described only late responses >100 ms as pathological. We showed that the time–frequency SPES analysis increases specificity for the seizure onset zone when incorporating signals in the high-frequency oscillation range. This does not rule out that better sensitivity and specificity can be reached by investigating early responses to local stimulation. In this retrospective study, such an analysis could not have been performed because of saturation of the amplifiers at the site of stimulation. New equipment that does not have this drawback will allow the important study of such early pathological high-frequency oscillation responses, also in conjunction with a thorough analysis of the early normal responses that have been described by Valentín *et al.* (2002).

Clinical findings

Although our population represents a small and heterogeneous group, our results resemble those by Valentín *et al.* (2002, 2005a, b) and by Flanagan *et al.* (2009) on the one hand, and recent high-frequency oscillation research on the other. They showed that delayed spike-like responses on SPES and spontaneous high-frequency oscillations are related to the seizure onset zone and surgical outcome (Urrestarazu *et al.*, 2007; Jacobs *et al.*, 2008). Our results suggest that time–frequency SPES evoked fast ripples may have the same clinical value. We could evoke ripples and fast ripples in all our patients, suggesting that evoked high-frequency oscillations, like spontaneous high-frequency oscillations, mark epileptogenicity independent of underlying pathology (Jacobs *et al.*, 2010), although we, like others, have no normal controls.

As noted by Lesser *et al.* (2010), ripples and fast ripples may occur simultaneously at sites separated by centimetres (Jacobs *et al.*, 2008; Crépon *et al.*, 2010), which might explain why some patients have a poor post-surgical outcome (Fig. 5 and Patients 9, 10 and 12 in Supplementary material). Partial removal of the epileptogenic zone might have been the case (Lesser *et al.*, 2010).

Time–frequency SPES results in two out of nine patients with follow-up were remarkable in that spikes, ripples, or fast ripples were found in areas that were not removed, while these patients (Patients 8 and 11) had a good post-surgical outcome. For Patient 8, no seizure onset zone was found, so grids were not in the right place. In this case, the resection was based on an assumption of a deep focus not covered by electrodes, corresponding with a lesion, and interpretation of the seizure spreading pattern. In Patient 11, an exceptionally low number of electrode pairs (16) were stimulated. Although these patients fared well, it is still possible that their epilepsy has not been cured, because both remained under medication. This is a problem for all studies of surgical outcome in which follow-up is limited to 1 year and medication withdrawal is not the endpoint (Wieser *et al.*, 2001).

Time–frequency SPES showed a decrease in sensitivity for ripples and fast ripples. Fast ripples demonstrated the lowest sensitivity for the seizure onset zone. Electrode coverage in subdural chronic electrocorticography may be insufficient to pick up all evoked responses. Also, incomplete coverage may result in defining a seizure onset zone, whereas seizure activity started in unexplored areas (including the depth of a sulcus). Of course, it should be kept in mind that seizure onset zone may not always predict the epileptogenic zone (Arzimanoglu and Kahane, 2008).

A weakness of our study may be that the clinicians taking care of the patients, although unaware of time–frequency SPES results, had the SPES data available, which were treated in the traditional way, i.e. by visual analysis restricted to the occurrence of evoked spikes, in line with the recommendations by Valentín *et al.* (2002). We did not comprehensively compare this type of visual evaluation with our time–frequency SPES classification of the evoked spike frequency range.

Our first results are encouraging in patients who are among the most difficult in epilepsy surgery. In the Netherlands, the work-up of epilepsy surgery is traditionally non-invasive and subdural

electrode implantations are restricted to a small number of the most complex patients (Leijten *et al.*, 2006). In patients who are implanted, the number of implanted electrodes tends to be high. We do not know how the number of electrode pairs stimulated, or the cortical surface area stimulated, relates to the number of false negative responses. The trends we show need to be confirmed in a larger and more general group of patients with focal refractory epilepsy. To establish the validity of time–frequency SPES as a method for delineation of the epileptogenic zone would ideally require a prospective study. Then the influence of time–frequency SPES results on resection and long-term outcome after medication withdrawal can be compared with outcome without time–frequency SPES results available to surgical decision making.

Implications and future directions

Time–frequency SPES is a promising method to assist controlled delineation of the epileptic focus. Its major advantage is that it covers the full spectrum of events (spikes, ripples and fast ripples) that can be used to delineate the epileptogenic cortex without depending on their spontaneous occurrence and the occurrence of seizures. The inclusion of high-frequency oscillatory responses in time–frequency SPES seems to increase specificity of the method and mirrors the experiences with spontaneous high-frequency oscillations compared with interictal spikes. A next step would be to automate interpretation of the ERSP images, with which time–frequency SPES could become a time-efficient way to use SPES in clinical practice. Time–frequency SPES could be used to reduce the region of interest and grid placement in invasive chronic electrocorticography monitoring, and even in acute electrocorticography for tailoring surgery intraoperatively. Larger, preferably prospective studies are needed to establish the full clinical potential.

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Supplementary material

Supplementary material is available at *Brain* online.

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